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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C12N 5/02, 5/06, 15/00, 15/64, C07H 21/04

A1

(11) International Publication Number: WO 98/14614

(43) International Publication Date: 9 April 1998 (09.04.98)

(21) International Application Number: PCT/US97/17791

(22) International Filing Date: 3 October 1997 (03.10.97)

(30) Priority Data:

08/726,867	4 October 1996 (04.10.96)	US
08/728,963	11 October 1996 (11.10.96)	US
08/907,598	8 August 1997 (08.08.97)	US

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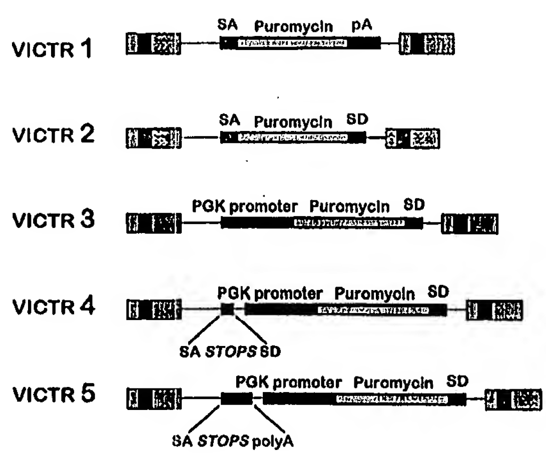
(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: AN INDEXED LIBRARY OF CELLS CONTAINING GENOMIC MODIFICATIONS AND METHODS OF MAKING AND UTILIZING THE SAME



(57) Abstract

Methods and vectors (both DNA and retroviral) are provided for the construction of a Library of mutated cells. The Library will preferably contain mutations in essentially all genes present in the genome of the cells. The nature of the Library and the vectors allow for methods of screening for mutations in specific genes, and for gathering nucleotide sequence data from each mutated gene to provide a database of tagged gene sequences. Such a database provides a means to access the individual mutant cell clones contained in the Library. The invention includes the described Library, methods of making the same, and vectors used to construct the Library. Methods are also provided for accessing individual parts of the Library either by sequence or by pooling and screening. The invention also provides for the generation of non-human transgenic animals which are mutant for specific genes as isolated and generated from the cells of the Library.

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AN INDEXED LIBRARY OF CELLS CONTAINING GENOMIC MODIFICATIONS AND METHODS OF MAKING AND UTILIZING THE SAME

The present application claims priority to U.S. Applications Ser. Nos. 08/726,867, filed October 4, 1996, 08/728,963, filed October 11, 1996, and 08/907,598, filed August 8, 1997, the disclosures of which are herein incorporated by reference.

1.0. FIELD OF THE INVENTION

The invention relates to an indexed library of genetically altered cells and methods of organizing the cells into an easily manipulated and characterized Library. The invention also relates to methods of making the library, vectors for making insertion mutations in genes, methods of gathering sequence information from each member clone of the Library, and methods of isolating a particular clone of interest from the Library.

2.0. BACKGROUND OF THE INVENTION

The general technologies of targeting mutations into the genome of cells, and the process of generating mouse lines from genetically altered embryonic stem (ES) cells with specific genetic lesions are well known (Bradley, 1991, Cur. Opin. Biotech. *2*:823-829). A random method of generating genetic lesions in cells (called gene, or promoter, trapping) has been developed in parallel with the targeted methods of genetic mutation (Allen *et al.*, 1988 Nature *333(6176)*:852-855; Brenner *et al.*, 1989, Proc. Natl. Acad. Sci. U.S.A. *86(14)*:5517-5521; Chang *et al.*, 1993, Virology *193(2)*:737-747; Friedrich and Soriano, 1993, Insertional mutagenesis by retroviruses and promoter traps in embryonic stem cells, p. 681-701. *In* Methods Enzymol., vol. *225.*, P. M. Wassarman and M. L. DePamphilis (ed.), Academic Press, Inc., San Diego; Friedrich and Soriano, 1991, Genes Dev. *5(9)*:1513-1523; Gossler *et al.*, 1989, Science *244(4903)*:463-465; Kerr *et al.*, 1989, Cold Spring Harb. Symp. Quant. Biol. *2*:767-776; Reddy *et al.*, 1991, J Virol. *65(3)*:1507-1515; Reddy *et al.*, 1992,

Proc. Natl. Acad. Sci. U.S.A. *89(15)*:6721-6725; Skarnes *et al.*, 1992, Genes Dev. *6(6)*:903-918; von Melchner and Ruley, 1989, J. Virol. *63(8)*:3227-3233; Yoshida *et al.*, 1995, Transgen. Res. 4:277-287). Gene trapping provides a means to create a collection of random mutations by inserting fragments of DNA into transcribed genes. Insertions into transcribed genes are selected over the background of total insertions since the mutagenic DNA encodes an antibiotic resistance gene or some other selectable marker. The selectable marker lacks its own promoter and enhancer and must be expressed by the endogenous sequences that flank the marker after it has integrated. Using this approach, transcription of the selectable marker is activated and the cell gene is concurrently mutated. This type of strict selection makes it possible to easily isolate thousands of ES cell colonies, each with a unique mutagenic insertion.

Collecting mutants on a large-scale has been a powerful genetic technique commonly used for organisms which are more amenable to such analysis than mammals. These organisms, such as *Drosophila melanogastor*, yeast *Saccharomyces cerevisiae*, and plants such as *Arabadopsis thalia* are small, have short generation times and small genomes (Bellen *et al.*, 1989, Genes Dev. *3(9)*:1288-1300; Bier *et al.*, 1989, Genes Dev. *3(9)*:1273-1287; Hope, 1991, Develop. *113(2)*:399-408. These features allow an investigator to rear many thousands or millions of different mutant strains without requiring unmanageable resources. However, these type of organisms have only limited value in the study of biology relevant to human physiology and health. It is therefore important to have the power of large-scale genetic analysis available for the study of a mammalian species that can aid in the study of human disease. Given that the entire human genome is presently being sequenced, the comprehensive genetic analysis of a related mammalian species will provide a means to determine the function of genes cloned from the human genome. At present, rodents, and particularly mice, provide the best model for genetic manipulation and analysis of mammalian

physiology.

Gene trapping has been used as an analytical tool to identify genes and regulatory regions in a variety of animal cell types. One system that has proved particularly useful is based on the use of ROSA (reverse orientation splice acceptor) retroviral vectors (Friedrich and Soriano, 1991 and 1993).

The ROSA system can generate mutations that result in a detectable homozygous phenotype with a high frequency. About 50% of all the insertions caused embryonic lethality. The specifically mutated genes may easily be cloned since the gene trapping event produces a fusion transcript. This fusion transcript has trapped exon sequences appended to the sequences of the selectable marker allowing the latter to be used as a tag in polymerase chain reaction (PCR)-based protocols, or by simple cDNA cloning. Examples of genes isolated by these methods include a transcription factor related to human TEF-1 (transcription enhancer factor-1) which is required in the development of the heart (Chen *et al.*, 1994, Genes Devel. *8*:2293-2301. Another (spock), is distantly related to yeast genes encoding secretion proteins and is important during gastrulation.

The above experiments have established that the ROSA system is an effective analytical tool for genetic analysis in mammals. However, the structure of many ROSA vectors selects for the "trapping" of 5′ exons which, in many cases, do not encode proteins. Such a result is adequate where one wishes to identify and eventually clone control (i.e., promoter or enhancer) sequences, but is not optimal where the generation of insertion-inactivated null mutations is desired, and relevant coding sequence is needed. Thus, the construction of large-scale mutant (preferably null mutant) libraries requires the use of vectors that have been designed to select for insertion events that have occurred within the coding region of the mutated genes as well as vectors that are not limited to detecting insertions into expressed genes.

3.0. SUMMARY OF THE INVENTION

An object of the present invention is to provide a set of genetically altered cells (the 'Library'). The genetic alterations are of sufficient randomness and frequency such that the combined population of cells in the Library represent mutations in essentially every gene found in the cell's genome. The Library is used as a source for obtaining specifically mutated cells, cell lines derived from the individually mutated cells, and cells for use in the production of transgenic non-human animals.

A further object is to provide the vectors, both DNA and retroviral based, that may be used to generate the Library. Typically, at least two distinct vector designs will be used in order to mutate genes that are actively expressed in the target cell, and genes that are not expressed in the target cell. Combining the mutant cells obtained using both types of vectors best ensures that the Library provides a comprehensive set of gene mutations.

A particularly useful vector class contemplated by the present invention includes a vector for inserting foreign exons into animal cell transcripts that comprises a selectable marker, a promoter element operatively positioned 5' to the selectable marker, a splice donor site operatively positioned 3' to the selectable marker, and a second mutagenic foreign polynucleotide sequence located upstream from the promoter element that disrupts, or otherwise "poisons", the splicing or read-through expression of the endogenous cellular transcript. Typically, the mutagenic foreign polynucleotide sequence may incorporate a polyadenylation (pA) site, a nested set of stop codons in each of the three reading frames, splice acceptor and splice donor sequences in operable combination, a mutagenic exon, or any mixture of mutagenic features that effectively prevent the expression of the cellular gene. For example, a polyadenylation sequence may be incorporated in addition to or in lieu of the splice donor sequence. A preferred organization for the mutagenic polynucleotide sequence

comprises a polyadenylation site positioned upstream from a selectable marker which is in turn located upstream from a splice acceptor sequence. Preferably, such a vector does not comprise a transcription terminator or polyadenylation site operatively positioned relative to the coding region of the selectable marker, and shall not comprise a splice acceptor site operatively positioned between the promoter element and the initiation codon of said selectable marker.

An additional vector contemplated by the present invention is designed to replace the normal 3′ end of an animal cell transcript with a foreign exon. Such a vector shall generally be engineered to comprise a selectable marker, a splice acceptor site operatively positioned upstream (5′) from the initiation codon of the selectable marker, and a polyadenylation site operatively positioned downstream (3′) from the termination codon (3′ end) of the selectable marker. Preferably, the vector will not comprise a promoter element operatively positioned upstream from the coding region of the selectable marker, and will not comprise a splice donor sequence operatively positioned between the 3′ end of the coding region of the selectable marker and the polyadenylation site.

Yet another vector contemplated by the present invention is a vector designed to insert a mutagenic foreign polynucleotide sequence within an animal cell transcript (i.e., the foreign polynucleotide sequence is flanked on both sides by endogenous exons). As described above, the mutagenic foreign polynucleotide sequence may be any sequence that disrupts the normal expression of the gene into which the vector has integrated. Optionally, the vector may additionally incorporate a selectable marker, a splice acceptor site operatively positioned 5′ to the initiation codon of the selectable marker, a splice donor site operatively positioned 3′ to said selectable marker. Preferably, this vector shall not comprise a polyadenylation site operatively positioned 3′ to the coding region of said selectable marker, and shall not comprise a promoter element

operatively positioned 5′ to the coding region of said selectable marker.

An additional embodiment of the present invention is a library of genetically altered cells that have been treated to stably incorporate one or more types of the vectors described above. The presently described library of cultured animal cells may be made by a process comprising the steps of treating (*i.e.*, infecting, transfecting, retrotransposing, or virtually any other method of introducing polynucleotides into a cell) a population of cells to stably integrate a vector that mediates the splicing of a foreign exon internal to a cellular transcript, transfecting another population of cells to stably integrate a vector that mediates the splicing of a foreign exon 5′ to an exon of a cellular transcript, and selecting for transduced cells that express the products encoded by the foreign exons.

Alternatively, an additional embodiment of the present invention describes a mammalian cell library made by a method comprising the steps of: transfecting a population of cells with a vector capable of expressing a selectable marker in the cell only after the vector inserts into the host genome; transfecting or infecting a population of cells with a vector containing a selectable marker that is substantially only expressed by cellular control sequences (after the vector integrates into the host cells genome); and growing the transfected cells under conditions that select for the expression of the selectable marker.

In an additional embodiment of the present invention, the two populations of transfected cells will be individually grown under selective conditions, and the resulting mutated population of cells collectively comprises a substantially comprehensive library of mutated cells.

In an additional embodiment of the present invention, the individual mutant cells in the library are separated and clonally expanded. Additionally, the clonally expanded mutant cells may then be analyzed to ascertain the DNA

sequence, or partial DNA sequence of the mutated host gene.

The presently described methods of making, organizing, and indexing libraries of mutated animal cells are also broadly applicable to virtually any eukaryotic cells that may be genetically manipulated and grown in culture.

The invention provides for sequencing every gene mutated in the Library. The resulting sequence database subsequently serves as an index for the library. In essence, every cell line in the Library is individually catalogued using the partial sequence information. The resulting sequence is specific for the mutated gene since the present methods are designed to obtain sequence information from exons that have been spliced to the marker sequence. Since the coverage of the mutagenesis is preferably the entire set of genes in the genome, the resulting Library sequence database contains sequence from essentially every gene in the cell. From this database, a gene of interest can be identified. Once identified, the corresponding mutant cell may be withdrawn from the Library based on cross reference to the sequence data.

An additional embodiment of the invention provides for methods of isolating mutations of interest from the Library. Two methods are proposed for obtaining individual mutant cell lines from the Library. The first provides a scheme where clones of the cells generated using the above vectors are pooled into sets of defined size. Using the procedure described below which utilizes reverse transcription (RT) and polymerase chain reaction (PCR), a cell line with a mutation in a gene whose sequence is partly or wholly known is isolated from organized sets of these pools. A few rounds of this screening procedure results in the isolation of the desired individual cell line.

A second procedure involves the sequencing of regions flanking the vector insertion sites in the various cells in the library. The sequence database generated from these data effectively constitutes an index of the clones in the library that may be used to identify cells having mutations in

specific genes.

4.0. DESCRIPTION OF THE FIGURES

Figure 1. Shows a diagrammatic representation of 5 different vectors that are generally representative of the type of vectors that may be used in the present invention.

Figure 2. Shows a general strategy for identifying "trapped" cellular sequences by PCR analysis of the cellular exons that flank the foreign intron introduced by the VICTR 2 vector.

Figure 3 shows a PCR based strategy for identifying tagged genes by chromosomal location.

Figure 4. Is a diagrammatic representation of a strategy of identifying or indexing the specific clones in the library via PCR analysis and sequencing of mRNA samples obtained from the cells in the library.

Figure 5. Is a diagrammatic representation of a method of isolating positive clones by screening pooled mutant cell clones.

Figure 6. Partial nucleic acid or predicted amino acid sequence data from 9 clones (OST1-9) isolated using the described techniques aligned with similar sequences from previously characterized genes.

Figure 7. Provides a diagrammatic representation of VICTRs 3 and 20 as well as the transcripts that result after integration into a hypothetical region of the target cell genome (*i.e.*, "Wildtype Locus).

Figure 8. Provides a representative list of a portion of the known genes that have been identified using the disclosed methods and technology.

5.0. DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a novel indexed library containing a substantially comprehensive set of mutations in the host cell genome, and methods of making and using the same. The presently described Library comprises as a set of cell clones that each possess at least one mutation (and preferably a single mutation) caused by the insertion of DNA that is foreign to the cell. For the purposes of the present invention, "foreign" polynucleotide sequences can be any sequences that are newly introduced to a cell, do not naturally occur in the cell at the engineered region of the chromosome, or occur in the cell but are not organized to provide an identical function to that provided in the engineered vector.

The particularly novel features of the Library include the methods of construction, and indexing. To index the library, the mutant cells of the library are clonally expanded and each mutated gene is at least partially sequenced. The Library thus provides a novel tool for assessing the specific function of a given gene. The insertions cause a mutation which allow for essentially every gene represented in the Library to be studied using genetic techniques either *in vitro* or *in vivo* (via the generation of transgenic animals). For the purposes of the present invention, the term "essentially every gene" shall refer to the statistical situation where there is generally at least about a 70 percent probability that the genomes of cells used to construct the library collectively contain at least one inserted vector sequence in each gene, preferably a 85 percent probability, and more specifically at least about a 95 percent probability as determined by a standard Poisson distribution.

Also for the purposes of the present invention the term "gene" shall refer to any and all discrete coding regions of the cell's genome, as well as associated noncoding and regulatory regions. Additionally, the term operatively positioned shall refer to the control elements or genes that

are provided with the proper orientation and spacing to provide the desired or indicated functions of the control elements or genes.

For the purposes of the present invention, a gene is "expressed" when a control element in the cell mediates the production of functional or detectable levels of mRNA encoded by the gene, or a selectable marker inserted therein. A gene is not expressed where the control element in the cell is absent, has been inactivated, or does not mediate the production of functional or detectable levels of mRNA encoded by the gene, or a selectable marker inserted therein.

5.1. <u>Vectors used to build the Library</u>

A number of investigators have developed gene trapping vectors and procedures for use in mouse and other cells (Allen *et al.*, 1988; Bellen *et al.*, 1989, Genes Dev. *3(9)*:1288-1300; Bier *et al.*, 1989, Genes Dev. *3(9)*:1273-1287; Bonnerot *et al.*, 1992, J Virol. *66(8)*:4982-4991; Brenner *et al.*, 1989; Chang *et al.*, 1993; Friedrich and Soriano, 1993; Friedrich and Soriano, 1991; Goff, 1987, Methods Enzymol. *152*:469-481; Gossler *et al.*; Hope, 1991; Kerr *et al.*, 1989; Reddy *et al.*, 1991; Reddy *et al.*, 1992; Skarnes *et al.*, 1992; von Melchner and Ruley; Yoshida *et al.*, 1995). The gene trapping system described in the present invention is based on significant improvements to the published SA (splice acceptor) DNA vectors and the ROSA (reverse orientation, splice acceptor) retroviral vectors (Chen *et al.*, 1994; Friedrich and Soriano, 1991 and 1993). The presently described vectors also use a selectable marker called *βgeo*. This gene encodes a protein which is a fusion between the β-galactosidase and neomycin phosphotransferase proteins. The presently described vectors place a splice acceptor sequence upstream from the *βgeo* gene and a poly-adenylation signal sequence downstream from the marker. The marker is integrated after transfection by, for example, electroporation (DNA vectors), or retroviral infection, and gene trap events are selected based on resistance to G418

resulting from activation of *βgeo* expression by splicing from the endogenous gene into the ROSA splice acceptor. This type of integration disrupts the transcription unit and preferably results in a null mutation at the locus.

Although gene trapping has proven a useful analytical tool, the present invention contemplates gene trapping on a large scale. The vectors utilized in the present invention have been engineered to overcome the shortcomings of the early gene trap vector designs, and to facilitate procedures allowing high throughput. In addition, procedures are described that allow the rapid and facile acquisition of sequence information from each trapped cDNA which may be adapted to allow complete automation. These latter procedures are also designed for flexibility so that additional molecular information can easily be obtained subsequently. The present invention therefore incorporates gene trapping into a larger and unique tool. A specially organized set of gene trap clones that provide a novel and powerful new tool of genetic analysis.

The presently described vectors are superficially similar to the ROSA family of vectors, but constitute significant improvements and provide for additional features that are useful in the construction and indexing of the Library. Typically, gene trapping vectors are designed to detect insertions into transcribed gene regions within the genome. They generally consist of a selectable marker whose normal expression is handicapped by exclusion of some element required for proper transcription. When the vector integrates into the genome, and acquires the necessary element by juxtaposition, expression of the selectable marker is activated. When such activation occurs, the cell can survive when grown in the appropriate selective medium which allows for the subsequent isolation and characterization of the trapped gene. Integration of the gene trap generally causes the gene at the site of integration to be mutated.

Some gene trapping vectors have a splice acceptor preceding a selectable marker and a poly-adenylation signal

following the selectable marker, and the selectable marker gene has its own initiator ATG codon. Using this arrangement, the fusion transcripts produced after integration generally only comprise exons 5′ to the insertion site to the known marker sequences. Where the vector has inserted into the 5′ region of the gene, it is often the case that the only exon 5′ to the vector is a non-coding exon. Accordingly, the sequences obtained from such fusions do not provide the desired sequence information about the relevant gene products. This is because untranslated sequences are generally less well conserved than coding sequences.

To compensate for the short-comings of earlier vectors, the vectors of the present invention have been designed so that 3′ exons are appended to the fusion transcript by replacing the poly-adenylation and transcription termination signals of earlier ROSA vectors with a splice donor (SD) sequence. Consequently transcription and splicing generally results in a fusion between all or most of the endogenous transcript and the selectable marker exon, for example *βgeo*, neomycin (*neo*) or puromycin (*puro*). The exon sequences immediately 3′ to the selectable marker exon may then be sequenced and used to establish a database of expressed sequence tags. The presently described procedures will typically provide approximately 200 nucleotides of sequence, or more. These sequences will generally be coding and therefore informative. The prediction that the sequence obtained will be from coding region is based on two factors. First, gene trap vectors are generally found near the 5′ end of the gene immediately after untranslated exons because the method selects for integration events that place the initiator ATG of the selectable marker as the first encountered, and thus used, for translation. Second, mammalian transcripts have short 5′ untranslated regions (UTRs) which are typically between 50 and 150 nucleotides in length.

The obtained sequence information also provides a ready source of probes that may be used to isolate the full-length

gene or cDNA from the host cell, or as heterologous probes for the isolation of homologous genes in other species.

Internal exons in mammalian transcripts are generally quite small, on the average 137 bases with few over 300 bases. Consequently, a large internal exon may be spliced less efficiently. Thus, the presently described vectors have been designed to sandwich relatively small selectable markers (for example: *neo* ,~800 bases, or a smaller drug resistance gene such as *puro* ,~600 bases) between the requisite splicing elements to produce relatively small exons. Exons of this size are more typical of mammalian exons and do not present undue problems for the splicing machinery of the cell. Such a design consideration is novel to the presently disclosed gene trapping vectors. Accordingly, an additional embodiment of the claimed vectors is that the respective splice acceptor and splice donor sites are engineered such that they are operatively positioned close to the ends of the selectable marker coding region (the region spanning from the initiation codon to the termination codon). Generally, the splice acceptor or splice donor sequences shall appear within about 80 bases from the nearest end of the selectable marker coding region, preferably within about 50 bases from the nearest end of the coding region, more preferably within about 30 bases from the nearest end of the coding regions and specifically within about 20 bases of the nearest end of the selectable marker coding region.

The new vectors are represented in retroviral form in Figure 1. They are used by infecting target cells with retroviral particles such that the proviruses shown in the schematic can be found in the genome of the target. These vectors are called VICTR which is an acronym for "viral constructs for trapping".

The presently described retroviral vectors may be used in conjunction with retroviral packaging cell lines such as those described in U.S. Patent No. 5,449,614 ("'614 patent") issued September 12, 1995, herein incorporated by reference. Where non-mouse animal cells are to be used as targets for

generating the described libraries, packaging cells producing retrovirus with amphotropic envelopes will generally be employed to allow infection of the host cells.

The mutagenic gene trap DNA may also be introduced into the target cell genome by various transfection techniques which are familiar to those skilled in the art such as electroporation, lipofection, calcium phosphate precipitation, infection, retrotransposition, and the like. Examples of such techniques may be found in Sambrook *et al.* (1989) Molecular Cloning Vols. I-III, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, and Current Protocols in Molecular Biology (1989) John Wiley & Sons, all Vols. and periodic updates thereof, herein incorporated by reference. The transfected versions of the retroviral vectors are typically plasmid DNA molecules containing DNA cassettes comprising the described features between the retroviral LTRs.

The vectors VICTR 1 and 2 (Fig. 1) are designed to trap genes that are transcribed in the target cell. To trap genes that are not expressed in the target cell, gene trap vectors such as VICTR 3, 4 and 5 (described below) are provided. These vectors have been engineered to contain a promoter element capable of initiating transcription in virtually any cell type which is used to transcribe the coding sequence of the selectable marker. However, in order to get proper translation of the marker product, and thus render the cell resistant to the selective antibiotic, a polyadenylation signal and a transcription termination sequence must be provided. Vectors VICTR 3 through 5 are constructed such that an effective polyadenylation signal can only be provided by splicing with an externally provided downstream exon that contains a poly-adenylation site. Therefore, since the selectable marker coding region ends only in a splice donor sequence, these vectors must be integrated into a gene in order to be properly expressed. In essence, these vectors append the foreign exon encoding the marker to the 5' end of an endogenous transcript. These events will tag genes and

create mutations that are used to make clones that will become part of the Library.

With the above design considerations, the VICTR series of vectors, or similarly designed and constructed vectors, have the following features. VICTR 1 is a terminal exon gene trap. VICTR 1 does not contain a control region that effectively mediates the expression of the selectable marker gene. Instead, the coding region of the selectable marker contained in VICTR 1, in this case encoding puromycin resistance (but which can be any selectable marker functional in the target cell type), is preceded by a splice acceptor sequence and followed by a polyadenylation addition signal sequence. The coding region of the *puro* gene has an initiator ATG which is downstream and adjacent to a region of sequence that is most favorable for translation initiation in eukaryotic cells - the so called Kozak consensus sequence (Kozak, 1989, J. Cell, Biol. *108(2)*:229-241). With a Kozak sequence and an initiator ATG, the *puro* gene in VICTR 1 is activated by integrating into the intron of an active gene, and the resulting fusion transcript is translated beginning at the puromycin initiation (ATG/AUG) codon. However, terminal gene trap vectors need not incorporate an initiator ATG codon. In such cases, the gene trap event requires splicing and the translation of a fusion protein that is functional for the selectable marker activity. The inserted puromycin coding sequence must therefore be translated in the same frame as the "trapped" gene.

The splice acceptor sequence used in VICTR 1 and other members of the VICTR series is derived from the adenovirus major late transcript splice site located at the intron 1/exon 2 boundary. This sequence contains a polypyrimidine stretch preceding the AG dinucleotide which denotes the actual splice site. The presently described vectors contemplate the use of any similarly derived splice acceptor sequence. Preferably, the splice acceptor site will only rarely, if ever, be involved in alternative splicing events.

The polyadenylation signal at the end of the *puro* gene is derived from the bovine growth hormone gene. Any similarly derived polyadenylation signal sequence could be used if it contains the canonical AATAAA and can be demonstrated to terminate transcription and cause a polyadenylate tail to be added to the engineered coding exons.

VICTR 2 is a modification of VICTR 1 in which the polyadenylation signal sequence is removed and replaced by a splice donor sequence. Like VICTR 1, VICTR 2 does not contain a control region that effectively mediates the expression of the selectable marker gene. Typically, the splice donor sequence to be employed in a VICTR series vector shall be determined by reference to established literature or by experimentation to identify which sequences properly initiate splicing at the 5′ end of introns in the desired target cell. The specifically exemplified sequence, AGGTAAGT, results in splicing occurring in between the two G bases. Genes trapped by VICTR 2 splice upstream exons onto the *puro* exon and downstream exons onto the end of the *puro* exon. Accordingly, VICTR 2 effectively mutates gene expression by inserting a foreign exon in-between two naturally occurring exons in a given transcript. Again, the *puro* gene may or may not contain a consensus Kozak translation initiation sequence and properly positioned ATG initiation codon. As discussed above, gene trapping by VICTR 1 and VICTR 2 requires that the mutated gene is expressed in the target cell line. By incorporating a splice donor into the VICTR traps, transcript sequences downstream from the gene trap insertion can be determined. As described above, these sequences are generally more informative about the gene mutated since they are more likely to be coding sequences. This sequence information is gathered according to the procedures described below.

VICTR 3, VICTR 4 and VICTR 5 are gene trap vectors that do not require the cellular expression of the endogenous trapped gene. The VICTR vectors 3 through 5 all comprise a

promoter element that ensures that transcription of the selectable marker would be found in all cells that have taken up the gene trap DNA. This transcription initiates from a promoter, in this case the promoter element from the mouse phosphoglycerate kinase (*PGK*) gene. However, since the constructs lack a polyadenylation signal there can be no proper processing of the transcript and therefore no translation. The only means to translate the selectable marker and get a resistant cell clone is by acquiring a polyadenylation signal. Since polyadenylation is known to be concomitant with splicing, a splice donor is provided at the end of the selectable marker. Therefore, the only positive gene trap events using VICTR 3 through 5 will be those that integrate into a gene's intron such that the marker exon is spliced to downstream exons that are properly polyadenylated. Thus genes mutated with the VICTR vectors 3 through 5 need not be expressed in the target cell, and these gene trap vectors can mutate all genes having at least one intron. The design of VICTR vectors 3 through 5 requires a promoter element that will be active in the target cell type, a selectable marker and a splice donor sequence. Although a specific promoter was used in the specific embodiments, it should be understood that appropriate promoters may be selected that are known to be active in a given cell type. Typically, the considerations for selecting the splice donor sequence are identical to those discussed for VICTR 2, *supra*.

VICTR 4 differs from VICTR 3 only by the addition of a small exon upstream from the promoter element of VICTR 4. This exon is intended to stop normal splicing of the mutated gene. It is possible that insertion of VICTR 3 into an intron might not be mutagenic if the gene can still splice between exons, bypassing the gene trap insertion. The exon in VICTR 4 is constructed from the adenovirus splice acceptor described above and the synthetic splice donor also described above. Stop codons are placed in all three reading frames in the exon, which is about 100 bases long. The stops would truncate the endogenous protein and presumably cause a

mutation.

A conceptually similar alternative design uses a terminal exon like that engineered into VICTR 5. Instead of a splice donor, a polyadenylation site is used to terminate transcription and produce a truncated message. Stops in all three frames are also provided to truncate the endogenous protein as well as the resulting transcript.

VICTR 20 is a modified version of VICTR 3 that incorporates a polyadenylation site 5′ to the PGK promoter, the IRESβgeo sequence (*i.e.*, foreign mutagenic polynucleotide sequence) 5′ to the polyadenylation site, and a splice acceptor site 5′ to the IRESβgeo coding region. VICTR 20 additionally incorporates, in operable combination, a pair of recombinase recognition sites that flank the PGKpuroSD cassette.

All of the traps of the VICTR series are designed such that a fusion transcript is formed with the trapped gene. For all but VICTR 1, the fusion contains cellular exons that are located 3′ to the gene trap insertion. All of the flanking exons may be sequenced according to the methods described in the following section. To facilitate sequencing, specific sequences are engineered onto the ends of the selectable marker (e.g., puromycin coding region). Examples of such sequences include, but are not limited to unique sequences for priming PCR, and sequences complementary to the standard M13 forward sequencing primer. Additionally, stop codons are added in all three reading frames to ensure that no anomalous fusion proteins are produced. All of the unique 3′ primer sequences are followed immediately by the synthetic 9 base pair splice donor sequence. This keeps the size of the exon comprising the selectable marker (*puro* gene) at a minimum to best ensure proper splicing, and positions the amplification and sequencing primers immediately adjacent to the flanking "trapped" exons to be sequenced as part of the construction of a Library database.

When any members of the VICTR series are constructed as retroviruses, the direction of transcription of the

selectable marker is opposite to that of the direction of the normal transcription of the retrovirus. The reason for this organization is that the transcription elements such as the polyadenylation signal, the splice sites and the promoter elements found in the various members of the VICTR series interfere with the proper transcription of the retroviral genome in the packaging cell line. This would eliminate or significantly reduce retroviral titers. The LTRs used in the construction of the packaging cell line are self-inactivating. That is, the enhancer element is removed from the 3' U3 sequences such that the proviruses resulting from infection would not have an enhancer in either LTR. An enhancer in the provirus may otherwise affect transcription of the mutated gene or nearby genes.

Since a 'cryptic' splice donor sequence is found in the inverted LTRs, this splice donor sequence has been removed from the VICTR vectors by site specific mutagenesis. It was deemed necessary to remove this splice donor so that it would not affect the trapping splicing events.

The present disclosure also describes vectors that incorporate a new way to conduct positive selection. VICTR 3 and VICTR 20 are two examples of such vectors. Both VICTR 3 and VICTR 20, contain PGKpuroSD which must splice into exons of gene that provide a polyadenylation addition sequence in order to allow expression of the puromycin selectable marker gene. When placed in a targeting vector, PGKpuroSD allows for positive selection when targeting takes place. In addition to providing positive selection, targeted events among resistant colonies are easy to identify by the 3' RACE protocols (see section 5.2.2., *infra*) used for Omnibank production. This automated process allows for the rapid identification of targeted events. It is important that unlike SAβgeo, PGKpuroSD does not require expression of the targeted gene in order to provide positive selection. In addition, VICTR 20 provides 2 potential positive selectable markers (puro and neo). The use of two selectable markers, when a gene is expressed, provides a means to increase the

targeting efficiency by requiring both selectable markers to function which is much more remote a possibility than having one selectable marker function unless there is a targeted event. The addition of a negative selection cassette to these vectors would only increase their targeting efficiency.

An additional feature that may be incorporated into the presently described vectors includes the use of recombinase recognition sequences. Bacteriophage P1 Cre recombinase and flp recombinase from yeast plasmids are two examples of site-specific DNA recombinase enzymes which cleave DNA at specific target sites (loxP sites for cre recombinase and frt sites for flp recombinase) and catalyze a ligation of this DNA to a second cleaved site. When a piece of DNA is flanked by 2 loxP or frt sites (*e.g.*, recombinase control elements) in the same orientation, the corresponding recombinase will cause the removal of the intervening DNA sequence. When a piece of DNA is flanked by loxP or frt sites in an indirect orientation, the corresponding recombinase will essentially activate the control elements to cause the intervening DNA to be flipped into the opposite orientation. These recombinases provide powerful approaches for manipulating DNA *in situ*.

Recombinases have important applications for gene trapping and the production of a library of trapped genes. When constructs containing PGKpuroSD are used to trap genes, the fusion transcript between puromycin and sequences of the trapped gene could result in some level of protein expression from the trapped gene if translational reinitiation occurs. Another important issue is that several reports suggest that the PGK promoter can affect the expression of nearby genes. These effects may make it difficult to determine gene function after a gene trap event since one could not discern whether a given phenotype is associated with the inactivation of a gene, or the transcription of nearby genes. Both potential problems are solved by exploiting recombinase activity. When PGKpuroSD is flanked by loxP, frt, or any other recombinase sites in the same orientation, the addition of the corresponding recombinase will result in the removal

of PGKpuroSD. In this way, effects caused by PGKpuroSD fusion transcripts, or the PGK promoter, are avoided.

Accordingly, a vector that may be particularly useful for the practice of the present invention is VICTR 20. This vector replaces the terminal exon of VICTR 5 with a splice acceptor located upstream from the βgeo gene which can be used for both LacZ staining and antibiotic selection. The fusion gene possesses its own initiator methionine and an internal ribosomal entry site (IRES) for efficient translation initiation. In addition, the PGK promoter and puromycin-splice donor sequences have been flanked by lox P recombination sites. This allows for the ability to both remove and introduce sequences at the integration site and is of potential value with regard to the manipulation of regions proximal to trapped target genes (Barinaga, Science 265:26-8, 1994). While this particular vector includes lox P recombination sites, the present invention is in no way limited to the use of this specific recombination site (Akagi *et al.*, Nucleic Acids Res 25:1766-73, 1997).

Another very important use of recombinases is to produce mutations that can be made tissue-specific and/or inducible. In the presently described vectors, the Saβgeo or SAIRESβgeo component provides the mutagenic function by "trapping" the normal splicing from preceding exons. If the SAβgeo is flanked by inverted loxP, frt, or any other recombinase sites, the addition of the corresponding recombinase results in the flipping of the SAβgeo sequence so that it no longer prevents the normal splicing of the cellular gene into which it is integrated. To make a gene trap tissue-specific or inducible one could produce the trap with SAβgeo in the reverse orientation and then provide recombinase activity only at the time and place where one wishes to remove the gene function. The use of tissue-specific or inducible recombinase constructs allows one to choose when and where one removes, or activates, the function of the targeted gene.

One method for practicing the inducible forms of recombinase mediated gene expression involves the use of

vectors that use inducible or tissue specific promoter/operator elements to express the desired recombinase activity. The inducible expression elements are preferably operatively positioned to allow the inducible control or activation of expression of the desired recombinase activity. Examples of such inducible promoters or control elements include, but are not limited to, tetracycline, metallothionine, ecdysone, and other steroid-responsive promoters, rapamycin responsive promoters, and the like (No *et al.*, Proc Natl Acad Sci USA 93:3345-51, 1996; Furth *et al.*, Proc Natl Acad Sci USA 91:9302-6, 1994). Additional control elements that can be used include promoters requiring specific transcription factors such as viral, particularly HIV, promoters. Vectors incorporating such promoters would only express recombinase activity in cells that express the necessary transcription factors.

The incorporation of recombinase sites into the gene trapping vectors highlights the value of using the described gene trap vectors to deliver specific DNA sequence elements throughout the genome. Although a variety of vectors are available for placing sequences into the genome, the presently described vectors facilitate both the insertion of the specific elements, and the subsequent identification of where sequence has inserted into the cellular chromosome. Additionally, the presently described vectors may be used to place recombinase recognition sites throughout the genome. The recombinase recognition sites could then be used to either remove or insert specific DNA sequences at predetermined locations.

Moreover, the described gene trap vectors can also be used to insert regulatory elements throughout the genome. Recent work has identified a number of inducible or repressible systems that function in the mouse. These include the rapamycin, tetracycline, ecdysone, glucocorticoid, and heavy metal inducible systems. These systems typically rely on placing DNA elements in or near a promoter. An inducible or repressible transcription factor

that can identify and bind to the DNA element may also be engineered into the cells. The transcription factor will specifically bind to the DNA element in either the presence or absence of a ligand that binds to the transcription factor and, depending on the structure of the transcription factor, it will either induce or repress the expression of the cellular gene into which the DNA elements have been inserted. The ability to place these inducible or repressible elements throughout the genome would increase the value of the library by adding the potential to regulate the expression of the trapped gene.

The vectors described also have important applications for the overexpression of genes or portions of genes to select for phenotypic effects. Currently, overexpression of cDNA libraries to look for genes or parts of genes with specific functions is a common practice. One example would be to overexpress genes or portions of genes to look for expression that causes loss of contact inhibition for cell growth as determined by growth in soft agar. This would allow the identification of genes or portions of genes that can act as oncogenes. Simple modifications of VICTR 20 would allow it to be used for these applications. For example, the addition of an internal ribosome entry site (IRES) 3' to the puromycin selectable marker and before the SD sequence, would result in the overexpression of sequences from the trapped downstream exons. In addition, the IRES could be modified by, for example, the addition of one or two nucleotides such that there could be 3 basic vectors that would allow expression of trapped exons in all three reading frames. In this way, genes could be trapped throughout the genome resulting in overexpression of genes, or portions thereof, to examine the cellular function of the trapped genes. This identification of function could be done by selecting for the function of interest (*i.e.*, growth in soft agar could result from the overexpression of potentially oncogenic genes). This technique would allow for the screening or selection of large numbers of genes, or portions thereof, by

overexpressing the genes and identifying cells displaying the phenotypes of interest. Additional assays could, for example, identify candidate tumor suppressor genes based on their ability, when overexpressed, to prevent growth in soft agar.

Given the fact that expression pattern information can provide insight into the possible functions of genes mutated by the current methods, another LTR vector, VICTR 6, has been constructed in a manner similar to VICTR 5 except that the terminal exon has been replaced with either a gene coding for β-galactosidase (βgal) or a fusion between β-gal and neomycin phosphotransferase (βgeo), each proceeded by a splice acceptor and followed by a polyadenylation signal. Endogenous gene expression and splicing of these markers into cellular transcripts and translation into fusion proteins will allow for increased mutagenicity as well as the delineation of expression through Lac Z staining.

An additional vector, VICTR 12, incorporates two separate selectable markers for the analysis of both integration sites and trapped genes. One selectable marker (e.g. puro) is similar to that for VICTRs 3 through 5 in that it contains a promoter element at its 5' end and a splice donor sequence 3'. This gene cassette is located in the LTRs of the retroviral vector. The other marker (neo) also contains a promoter element but has a polyadenylation signal present at the 3' end of the coding sequence and is positioned between the viral LTRs. Both selectable markers contain an initiator ATG for proper translation. The design of VICTR 12 allows for the assessment of absolute titer as assayed by the number of colonies resistant to antibiotic selection for the constitutively expressed marker possessing a polyadenylation signal. This titer can then be compared to that observed for gene-trapping and stable expression of the resistance marker flanked at its 3' end by a splice donor. These numbers are important for the calculation of gene trapping frequency in the context of both nonspecific binding by retroviral integrase and directed binding by chimeric

integrase fusions. In addition, it provides an option to focus on the actual integration sites through infection and selection for the marker containing the polyadenylation signal. This eliminates the need for the fusion protein binding to occur upstream and in the proximity of the target gene. Theoretically, any transcription factor binding sites present within the genome are targets for proximal integration and subsequent antibiotic resistance. Analysis of sequences flanking the LTRs of the retroviral vector should reveal canonical factor binding sites. In addition, by including the promoter/splice donor design of VICTR 3, gene-trapping abilities are retained in VICTR 12.

VICTR A is a vector which does not contain gene trapping constructs but rather a selectable marker possessing all of the required entities for constitutive expression including, but not limited to, a promoter element capable of driving expression in eukaryotic cells and a polyadenylation and transcriptional terminal signal. Similar to VICTR 12, downstream gene trapping is not necessary for successful selection using VICTR A. This vector is intended solely to select for successful integrations and serves as a control for the identification of transcription factor binding sites flanking the integrant as mentioned above.

Finally, VICTR B is similar to VICTR A in that it comprises a constitutively expressed selectable marker, but it also contains the bacterial β-lactamase ampicillin resistance selectable marker and a ColE1 origin of replication. These entities allow for the rapid cloning of sequences flanking the long terminal repeats through restriction digestion of genomic DNA from infected cells and ligation to form plasmid molecules which can be rescued by bacterial transformation, and subsequently sequenced. This vector allows for the rapid analysis of cellular sequences that contain putative binding sites for the transcription factor of interest.

Other vector designs contemplated by the present invention are engineered to include an inducible regulatory

elements such as tetracycline, ecdysone, and other steroid-responsive promoters (No *et al.*, Proc Natl Acad Sci USA 93:3345-51, 1996; Furth *et al.*, Proc Natl Acad Sci USA 91:9302-6, 1994). These elements are operatively positioned to allow the inducible control of expression of either the selectable marker or endogenous genes proximal to site of integration. Such inducibility provides a unique tool for the regulation of target gene expression.

All of the gene trap vectors of the VICTR series, with the exception of VICTRs A and B, are designed to form a fusion transcript between vector encoded sequence and the trapped target gene. All of the flanking exons may be sequenced according to the methods described in the following section. To facilitate sequencing, specific sequences are engineered onto the ends of the selectable marker (e.g., puromycin coding region). Examples of such sequences include, but are not limited to unique sequences for priming PCR, and sequences complementary to standard M13 sequencing primers. Additionally, stop codons are added in all three reading frames to ensure that no anomalous fusion proteins are produced. All of the unique 3' primer sequences are immediately followed by a synthetic 9 base pair splice donor sequence. This keeps the size of the exon comprising the selectable marker at a minimum to ensure proper splicing, and positions the amplification and sequencing primers immediately adjacent to the flanking trapped exons to be sequenced as part of the generation of the collection of cells representing mutated transcription factor targets.

Since a cryptic splice donor sequence is found in the inverted LTRs, this cryptic splice donor sequence has been removed from the VICTR vectors by site specific mutagenesis. It was deemed necessary to remove this splice donor so that it would not affect trapping associated splicing events.

When any members of the VICTR series are packaged into infectious virus, the direction of transcription of the selectable marker is opposite to that of the direction of the normal transcription of the retrovirus. The reason for this

organization is that the regulatory elements such as the polyadenylation signal, the splice sites and the promoter elements found in the various members of the VICTR series can interfere with the transcription of the retroviral genome in the packaging cell line. This potential interference may significantly reduce retroviral titers.

Although specific gene trapping vectors have been discussed at length above, the invention is by no means to be limited to such vectors. Several other types of vectors that may also be used to incorporate relatively small engineered exons into a target cell transcripts include, but are not limited to, adenoviral vectors, adenoassociated virus vectors, SV40 based vectors, and papilloma virus vectors. Additionally, DNA vectors may be directly transferred into the target cells using any of a variety of biochemical or physical means such as lipofection, chemical transfection, retrotransposition, electroporation, and the like.

Although, the use of specific selectable markers has been disclosed and discussed herein, the present invention is in no way limited to the specifically disclosed markers. Additional markers (and associated antibiotics) that are suitable for either positive or negative selection of eukaryotic cells are disclosed, *inter alia*, in Sambrook *et al*. (1989) Molecular Cloning Vols. I-III, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, and Current Protocols in Molecular Biology (1989) John Wiley & Sons, all Vols. and periodic updates thereof, as well as Table I of U.S. Patent No. 5,464,764 issued November 7, 1995, the entirety of which is herein incorporated by reference. Any of the disclosed markers, as well as others known in the art, may be used to practice the present invention.

5.2. The Analysis of Mutated Genes and Transcripts

The presently described invention allows for large-scale genetic analysis of the genomes of any organism for which there exists cultured cell lines. The Library may be constructed from any type of cell that can be transfected by

standard techniques or infected with recombinant retroviral vectors.

Where mouse ES cells are used, then the Library becomes a genetic tool able to completely represent mutations in essentially every gene of the mouse genome. Since ES cells can be injected back into a blastocyst and become incorporated into normal development and ultimately the germ line, the cells of the Library effectively represent a complete panel of mutant transgenic mouse strains (see generally, U.S. Patent No. 5,464,764 issued November 7, 1995, herein incorporated by reference).

A similar methodology may be used to construct virtually any non-human transgenic animal (or animal capable of being rendered transgenic). Such nonhuman transgenic animals may include, for example, transgenic pigs, transgenic rats, transgenic rabbits, transgenic cattle, transgenic goats, and other transgenic animal species, particularly mammalian species, known in the art. Additionally, bovine, ovine, and porcine species, other members of the rodent family, e.g. rat, as well as rabbit and guinea pig and non-human primates, such as chimpanzee, may be used to practice the present invention.

Transgenic animals produced using the presently described library and/or vectors are useful for the study of basic biological processes and diseases including, but not limited to, aging, cancer, autoimmune disease, immune disorders, alopecia, glandular disorders, inflammatory disorders, diabetes, arthritis, high blood pressure, atherosclerosis, cardiovascular disease, pulmonary disease, degenerative diseases of the neural or skeletal systems, Alzheimer's disease, Parkinson's disease, asthma, developmental disorders or abnormalities, infertility, epithelial ulcerations, and microbial pathogenesis (a relatively comprehensive review of such pathogens is provided, *inter alia*, in Mandell *et al.*, 1990, "Principles and Practice of Infectious Disease" 3rd. ed., Churchill Livingstone Inc., New York, N.Y. 10036, herein incorporated

by reference). As such, the described animals and cells are particularly useful for the practice of functional genomics.

5.2.1. Constructing a Library of Individually Mutated Cell Clones

The vectors described in the previous section were used to infect (or transfect) cells in culture, for example, mouse embryonic stem (ES) cells. Gene trap insertions were initially identified by antibiotic resistance (e.g., puromycin). Individual clones (colonies) were moved from a culture dish to individual wells of a multi-welled tissue culture plate (e.g. one with 96 wells). From this platform, the clones were be duplicated for storage and subsequent analysis. Each multi-well plate of clones was then processed by molecular biological techniques described in the following section in order to derive sequence of the gene that has been mutated. This entire process is presented schematically in Figure 4 (described below).

5.2.2. Identifying and Sequencing the Tagged Genes in the Library.

The relevant nucleic acid (and derived amino acid sequence information) will largely be obtained using PCR-based techniques that rely on knowing part of the sequence of the fusion transcripts (see generally, Frohman *et al.*, 1988, Proc. Natl. Acad. Sci. U.S.A. *85(23)*:8998-9000, and U.S. Patents Nos. 4,683,195 to Saiki *et al.*, and 4,683,202 to Mullis, which are herein incorporated by reference). Typically, such sequences are encoded by the foreign exon containing the selectable marker. The procedure is represented schematically in Figure 2 (3' RACE). Although each step of the procedure may be done manually, the procedure is also designed to be carried out using robots that can deliver reagents to multi well culture plates (e.g., but not limited to, 96-well plates).

The first step generates single stranded complementary DNA which is used in the PCR amplification reaction (Figure

2). The RNA substrate for cDNA synthesis may either be total cellular RNA or an mRNA fraction; preferably the latter. mRNA was isolated from cells directly in the wells of the tissue culture dish. The cells were lysed and mRNA was bound by the complementary binding of the poly-adenylate tail to a poly-thymidine-associated solid matrix. The bound mRNA was washed several times and the reagents for the reverse transcription (RT) reaction were added. cDNA synthesis in the RT reaction was initiated at random positions along the message by the binding of a random sequence primer (RS). This RS primer has approximately 6-9 random nucleotides at the 3′ end to bind sites in the mRNA to prime cDNA synthesis, and a 5′ tail sequence of known composition to act as an anchor for PCR amplification in the next step. There is therefore no specificity for the trapped message in the RT step. Alternatively, a poly-dT primer appended with the specific sequences for the PCR may be used. Synthesis of the first strand of the cDNA initiates at the end of each trapped gene. At this point in the procedure, the bound mRNA may be stored (at between about -70° C and about 4° C) and reused multiple times. Such storage is a valuable feature where one subsequently desires to analyze individual clones in more detail. The bound mRNA may also be used to clone the entire transcript using PCR-based protocols.

Specificity for the trapped, fusion transcript is introduced in the next step, PCR amplification. The primers for this reaction are complementary to the anchor sequence of the RS primer and to the selectable marker. Double stranded fragments between a fixed point in the selectable marker gene and various points downstream in the appended transcript sequence are amplified. It is these fragments which will become the substrates for the sequencing reaction. The various end-points along the transcript sequence were determined by the binding of the random primer during the RT reaction. These PCR products were diluted into the sequencing reaction mix, denatured and sequenced using a primer specific for the splice donor sequences of the gene

trap exon. Although, standard radioactively labeled nucleotides may be used in the sequencing reactions, sequences will typically be determined using standard dye terminator sequencing in conjunction with automated sequencers (e.g., ABI sequencers and the like).

Several fragments of various sizes may serve as substrates for the sequencing reactions. This is not a problem since the sequencing reaction proceeds from a fixed point as defined by a specific primer sequence. Typically, approximately 200 nucleotides of sequence were obtained for each trapped transcript. For the PCR fragments that are shorter than this, the sequencing reaction simply 'falls off' the end. Sequences further 3' were then covered by the longer fragments amplified during PCR. One problem is presented by the anchor sequences 'S' derived from the RS primer. When these are encountered during the sequencing of smaller fragments, they register as anomalous dye signals on the sequencing gels. To circumvent this potential problem, a restriction enzyme recognition site is included in the S sequence. Digestion of the double stranded PCR products with this enzyme prior to sequencing eliminates the heterologous S sequences.

5.2.3. Identifying the Tagged Genes by Chromosomal Location

Any individually tagged gene may also be identified by PCR using chromosomal DNA as the template. To find an individual clone of interest in the Library arrayed as described above, genomic DNA is isolated from the pooled clones of ES cells as presented in Figure 3. One primer for the PCR is anchored in the gene trap vector, e.g., a *puro* exon-specific oligonucleotide. The other primer is located in the genomic DNA of interest. This genomic DNA primer may consist of either (1) DNA sequence that corresponds to the coding region of the gene of interest, or (2) DNA sequence from the locus of the gene of interest. In the first case, the only way that the two primers used may be juxtaposed to

give a positive PCR results (e.g., the correct size double-stranded DNA product) is if the gene trap vector has inserted into the gene of interest. Additionally, degenerate primers may be used, to identify and isolate related genes of interest. In the second case, the only way that the two primers used may be juxtaposed to provide the desired PCR result is if the gene trap vector has inserted into the region of interest that contains the primer for the known marker.

For example, if one wishes to obtain ES cell clones from the library that contain mutated genes located in a certain chromosomal position, PCR primers are designed that correspond to the *puro* gene (the puro-anchored primer) and a primer that corresponds to a marker known to be located in the region of interest. Several different combinations of marker primers and primers that are located in the region of interest may also be used to obtain optimum results. In this manner, the mutated genes are identified by virtue of their location relative to sets of known markers. Genes in a particular chromosomal region of interest could therefore be identified. The marker primers could also be designed correspond to sequences of known genes in order to screen for mutations in particular genes by PCR on genomic DNA templates. While this method is likely to be less informative than the RT-PCR strategy described below, this technique would be useful as a alternative strategy to identify mutations in known genes. In addition, primers that correspond to sequence of known genes could be used in PCR reactions with marker-specific primers in order to identify ES cell clones that contain mutations in genes proximal to the known genes. The sensitivity of detection is adequate to find such events when positive clones are subsequently identified as described below in the RT-PCR strategy.

5.3. A Sequence Database Identifies Genes Mutated in the Library.

Using the procedures described above, approximately 200

to about 600 bases of sequence from the cellular exons appended to the selectable marker exon (e.g., *puro* exon in VICTR vectors) may be identified. These sequences provide a means to identify and catalogue the genes mutated in each clone of the Library. Such a database provides both an index for the presently disclosed libraries, and a resource for discovering novel genes. Alternatively, various comparisons can be made between the Library database sequences and any other sequence database as would be familiar to those practiced in the art.

The novel utility of the Library lies in the ability for a person to search the Library database for a gene of interest based upon some knowledge of the nucleic acid or amino acid sequence. Once a sequence is identified, the specific clone in the Library can be accessed and used to study gene function. This is accomplished by studying the effects of the mutation both *in vitro* and *in vivo*. For example, cell culture systems and animal models (i.e., transgenic animals) may be directly generated from the cells found in the Library as will be familiar to those practiced in the art.

Additionally, the sequence information may be used to generate a highly specific probe for isolating both genomic clones from existing data bases, as well as a full length cDNA. Additionally, the probe may be used to isolate the homologous gene from sufficiently related species, including humans. Once isolated, the gene may be over expressed, or used to generate a targeted knock-out vector that may be used to generate cells and animals that are homozygous for the mutation of interest. Such animals and cells are deemed to be particularly useful as disease models (i.e., cancer, genetic abnormalities, AIDS, etc.), for developmental study, to assay for toxin susceptibility or the efficacy of therapeutic agents, and as hosts for gene delivery and therapy experiments (e.g., experiments designed to correct a specific genetic defect *in vivo*).

5.4. Accessing Clones in the Library by a Pooling and Screening Procedure.

An alternative method of accessing individual clones is by searching the Library database for sequences in order to isolate a clone of interest from pools of library clones. The Library may be arrayed either as single clones, each with different insertions, or as sets of pooled clones. That is, as many clones as will represent insertions into essentially every gene in the genome are grown in sets of a defined number. For example, 100,000 clones can be arrayed in 2,000 sets of 50 clones. This can be accomplished by titrating the number of VICTR retroviral particles added to each well of 96-well tissue culture plates. Two thousand clones will fit on approximately 20 such plates. The number of clones may be dictated by the estimated number of genes in the genome of the cells being used. For example, there are approximately 100,000 genes in the genome of mouse ES cells. Therefore, a Library of mutations in essentially every gene in the mouse genome may be arrayed onto 20 96-well plates.

To find an individual clone of interest in the Library arrayed in this manner, reverse transcription-polymerase chain reactions (RT-PCR) are performed on mRNA isolated from pooled clones as presented in Figure 4. One primer for RT-PCR is anchored in the gene trap vector, i.e. a *puro* exon-specific oligonucleotide. The other primer is located in the cDNA sequence of a gene of interest. The only way that these two sequences can be juxtaposed to give a positive RT-PCR result (i.e. double stranded DNA fragment visible by agarose gel electrophoresis, as will be familiar to anyone practiced in the art) is by being present in a transcript from a gene trap event occurring in the gene of interest.

For example, if one wishes to obtain an ES cell clone with a mutation in the p53 gene, PCR primers are designed that correspond to the *puro* and p53 genes. If a VICTR trapping vector integrates into the p53 locus and results in the formation of a fusion mRNA, this mRNA may be detected by RT-PCR using these specifically designed primer pairs. The

sensitivity of detection is adequate to find such an event when positive cells are mixed with a large background of negative cells. The individual positive clones are subsequently identified by first locating the pool of 50 clones in which it resides. This process is described in Figure 5. The positive pool, once identified, is subsequently plated at limiting dilution (approximately 0.3 cells/well) such that individual clones may be isolated. To find the one positive event in 50 clones represented by this pool, individual clones are isolated and arrayed on a 96-well plate. By pooling in columns and rows, the positive well containing the positive clone can be identified with relatively few RT-PCR reactions.

In addition to RT-PCR, the pools may be screened by hybridization techniques (see generally Sambrook *et al.*, 1989, Molecular Cloning: H Laboratory Manual 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, and Current Protocols in Molecular Biology, 1995, Ausubel *et al.* eds., John Wiley and Sons). Specific PCR fragments are generated from the mutated genes essentially as described above for the sequencing protocols of the individual clones (first-strand synthesis using RT primed by a random or oligo dT primer that is appended to a specific primer binding site). The gene trap DNA is amplified from the primer sets in the *puro* gene and the specific sequences appended to the RT primer. If this were done with pools, the resulting pooled set of amplified DNA fragments could be arrayed on membranes and probed by radioactive, or chemically or enzymatically labeled, hybridization probes specific for a gene of interest. A positive radioactive result indicates that the gene of interest has been mutated in one of the clones of the positively-labeled pool. The individual positive clone is subsequently identified by PCR or hybridization essentially as outlined above.

Alternatively, a similar strategy may be used to identify the clone of interest from multiple plates, or any scheme where a two or three dimensional array (e.g., columns

and rows) of individual clones are pooled by row or by column. For example, 96 well plates of individual clones may be arranged adjacent to each other to provide a larger (or virtual/figurative) two dimensional grid (e.g., four plates may be arranged to provide a net 16x24 grid), and the various rows and columns of the larger grid may be pooled to achieve substantially the same result.

Similarly, plates may simply be stacked, literally or figuratively, or arranged into a larger grid and stacked to provide three dimensional arrays of individual clones. Representative pools from all three planes of the three dimensional grid may then be analyzed, and the three positive pools/planes may be aligned to identify the desired clone. For example, ten 96 well plates may be screened by pooling the respective rows and columns from each plate (a total of 20 pools) as well as pooling all of the clones on each specific plate (10 additional pools). Using this method, one may effectively screen 960 clones by performing PCR on only 30 pooled samples.

The example provided below is merely illustrative of the subject invention. Given the level of skill in the art, one may be expected to modify any of the above or following disclosure to produce insubstantial differences from the specifically described features of the present invention. As such, the following example is provided solely by way of illustration and is not included for the purpose of limiting the invention in any way whatsoever.

6.0. EXAMPLES

6.1. Use of VICTR Series Vectors to Construct a Mouse ES cell Gene Trap Library

VICTR 3 was used to gather a set of gene trap clones. A plasmid containing the VICTR 3 cassette was constructed by conventional cloning techniques and designed to employ the features described above. Namely, the cassette contained a *PGK* promoter directing transcription of an exon that encodes the *puro* marker and ends in a canonical splice donor

sequence. At the end of the puromycin exon, sequences were added as described that allow for the annealing of two nested PCR and sequencing primers. The vector backbone was based on pBluescript KS+ from Stratagene Corporation.

The plasmid construct linearized by digestion with Sca I which cuts at a unique site in the plasmid backbone. The plasmid was then transfected into the mouse ES cell line AB2.2 by electroporation using a BioRad Genepulser apparatus. After the cells were allowed to recover, gene trap clones were selected by adding puromycin to the medium at a final concentration of 3 μg/mL. Positive clones were allowed to grow under selection for approximately 10 days before being removed and cultured separately for storage and to determine the sequence of the disrupted gene.

Total RNA was isolated from an aliquot of cells from each of 18 gene trap clones chosen for study. Five micrograms of this RNA was used in a first strand cDNA synthesis reaction using the "RS" primer. This primer has unique sequences (for subsequent PCR) on its 5' end and nine random nucleotides or nine T (thymidine) residues on it's 3' end. Reaction products from the first strand synthesis were added directly to a PCR with outer primers specific for the engineered sequences of puromycin and the "RS" primer. After amplification, an aliquot of reaction products were subject to a second round of amplification using primers internal, or nested, relative to the first set of PCR primers. This second amplification provided more reaction product for sequencing and also provided increased specificity for the specifically gene trapped DNA.

The products of the nested PCR were visualized by agarose gel electrophoresis, and seventeen of the eighteen clones provided at least one band that was visible on the gel with ethidium bromide staining. Most gave only a single band which is an advantage in that a single band is generally easier to sequence. The PCR products were sequenced directly after excess PCR primers and nucleotides were removed by filtration in a spin column (Centricon-100, Amicon). DNA was

added directly to dye terminator sequencing reactions (purchased from ABI) using the standard M13 forward primer a region for which was built into the end of the *puro* exon in all of the PCR fragments. Thirteen of the seventeen clones that gave a band after the PCR provided readable sequence. The minimum number of readable nucleotides was 207 and some of the clones provided over 500 nucleotides of useful sequence.

Sample data from this set of clones is presented in Figure 6. Only a portion of sequence (nucleotide or putative amino acid) for 9 Library clones obtained by the methods described in this invention are presented. Under each sequence fragment in the figure is aligned a homologous sequence that was identified using the BLAST (basic local alignment search tool) search algorithm (Altschul *et al.*, 1990, J. Mol. Biol. *215*:403-410).

In addition to known sequences, many new genes were also identified. Each of these sequences is labeled "OST" for "Omnibank Sequence Tags." OMNIBANK™ shall be the trademark name for the Libraries generated using the disclosed technology.

These data demonstrate that the VICTR series vectors may efficiently trap genes, and that the procedures used to obtain sequence are reliable. With simple optimization of each step, it is presently possible to mutate every gene in a given population of cells, and obtain sequence from each of these mutated genes. The sample data provided in this example represents a small fraction of an entire Library. By simply performing the same procedures on a larger scale (with automation) a Library may be constructed that collectively comprises and indexes mutations in essentially every gene in the genome of the target cell.

Additional studies have used both VICTR 3 and VICTR 20. Like VICTR 3, VICTR 20 is exemplary of a family of vectors that incorporate two main functional units: a sequence acquisition component having a strong promoter element (phosphoglycerate kinase 1) active in ES cells that is fused

to the puromycin resistance gene coding sequence which lacks a polyadenylation sequence but is followed by a synthetic consensus splice donor sequence (PGKpuroSD); and 2) a mutagenic component that incorporates a splice acceptor sequence fused to a selectable, colorimetric marker gene and followed by a polyadenylation sequence (for example, SAβgeopA or SAIRESβgeopA). Also like VICTR 3, stop codons have been engineered into all three reading frames in the region between the 3' end of the selectable marker and the splice donor site. A diagrammatic description of structure and functions of VICTRs 3 and 20 is provided in Figure 7.

When VICTRs 3 and 20 were used in the commercial scale application of the presently disclosed invention, over 3,000 mutagenized ES cell clones were rapidly engineered and obtained. Sequence analysis obtained from these clones has identified a wide variety of both previously identified and novel sequences. A representative sampling of previously known genes that were identified using the presently described methods is provided in Figure 8. The power of the presently described invention as a genomics resource becomes apparent when one considers that the genes listed in Figure 8 were obtained and identified in less than a year whereas the references associated with the identification of the known genes span a period of roughly two decades. More importantly, the majority of the sequences thus far identified are novel, and, because of the functional aspects of the presently described ES cell system, the cellular and developmental functions of these novel sequences can be rapidly established.

7.0. Reference to Microorganism Deposits

The following plasmids have been deposited at the American Type Culture Collection (ATCC), Rockville, MD, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and Regulations thereunder (Budapest Treaty) and are thus maintained and made available according

to the terms of the Budapest Treaty. Availability of such plasmids is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The deposited cultures have been assigned the indicated ATCC deposit numbers:

Plasmid	ATCC No.
plex	97748
pExonII	97749
ppuro7	97750
ppuro5	97751
ppuro11	97752
ppuro10	97753

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 40, lines 5-25 of the description '

A. IDENTIFICATION OF DEPOSIT '

Further deposits are identified on an additional sheet '

Name of depositary institution '

American Type Culture Collection

Address of depositary institution (including postal code and country) '

12301 Parklawn Drive
Rockville, MD 20852
US

Date of deposit ' October 9, 1996 Accession Number ' 97748

B. ADDITIONAL INDICATIONS ' (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ' (if the indications are not all designated States)

D. SEPARATE FURNISHING OF INDICATIONS ' (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later ' (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the International application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau '

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

Accession No.	Date of Deposit
97749	October 9, 1996
97750	October 9, 1996
97751	October 9, 1996
97752	October 9, 1996
97753	October 9, 1996

CLAIMS

What is claimed is:

1. A library of cultured eucaryotic cells made by a process comprising the steps of:

a) treating a first group of cells to stably integrate a first vector that mediates the splicing of a foreign exon internal to a cellular transcript;

b) treating a second group of cells to stably integrate a second vector that mediates the splicing of a foreign exon 5' to an exon of a cellular transcript; and

c) selecting for transduced cells that express the products encoded by the foreign exons.

2. A library according to claim 1 wherein said treating is transfection.

3. A library according to claim 1 wherein said treating is by infection.

4. A library according to claim 1 wherein said treating is by retrotransposition.

5. A library according to any one of claims 1 through 4 wherein said cells are animal cells.

6. A library according to claim 5 wherein said animal is mammalian.

7. A library according to claim 6 wherein said cells are rodent cells.

8. The use of a mutated cell from a library according to claim 6 to generate a non-human transgenic animal.

9. A vector for replacing the 3' end of an animal cell transcript with a foreign exon, comprising:

a) a selectable marker;

b) a splice acceptor site operatively positioned 5′ to the initiation codon of said selectable marker;

c) a polyadenylation site operatively positioned 3′ to said selectable marker;

d) said vector not comprising a promoter element operatively positioned 5′ of the coding region of said selectable marker; and

e) said vector not comprising a splice donor sequence operatively positioned between the 3′ end of the coding region of said selectable marker and said polyadenylation site.

10. A vector for inserting foreign mutagenic polynucleotide sequence internal to animal cell transcripts, comprising:

a) a foreign exon;

b) a splice acceptor sequence operatively positioned 5′ to the foreign exon;

c) a splice donor site operatively positioned 3′ to said foreign exon;

d) a sequence comprising a nested set of stop codons in each of the three reading frames located between the 3′ end of said foreign exon and said splice donor site;

e) said vector not comprising a polyadenylation site operatively positioned 3′ to said foreign exon; and

f) said vector not comprising a promoter element operatively positioned 5′ to the coding region of said foreign exon.

11. A vector for attaching a foreign exon upstream from the 3′ end of an animal cell transcript, comprising:

a) a selectable marker;

b) a promoter element operatively positioned 5′ to said selectable marker;

c) a splice donor site operatively positioned 3′ to said selectable marker; and

d) said vector not comprising a transcription terminator or polyadenylation site operatively positioned relative to the coding region of said selectable marker; and

e) said vector not comprising a splice acceptor site operatively positioned between said promoter element and the initiation codon of said selectable marker.

12. A vector according to claim 11 wherein said vector additionally comprises a foreign mutagenic polynucleotide sequence located upstream from said promoter.

13. A vector according to claim 12 wherein said vector additionally comprises a splice acceptor operatively positioned upstream from said foreign mutagenic polynucleotide sequence.

14. A vector according to claim 13 wherein said foreign mutagenic polynucleotide sequence comprises a polyadenylation site.

15. A vector according to claim 14, wherein said foreign mutagenic polynucleotide sequence additionally comprises stop codons in all three reading frames.

16. A vector according to claim 12 in which a first recombinase recognition sequence is present upstream from said promoter and a second recombinase recognition sequence is present downstream from said promoter.

17. A vector according to any one of claims 9, 10, or 11 wherein said vector is a viral vector.

18. A vector according to claim 17 wherein said viral vector is a retroviral vector.

19. The use of a vector according to claim 9 to produce a library of mutated animal cells.

20. The use of a vector according to claim 10 to produce mutated animal cells.

21. The use of a vector according to claim 11 to produce mutated animal cells.

22. The use of a vector according to claim 11 to effect homologous recombination in an animal cell.

23. A stably transduced animal cell that incorporates a vector according to claim 16.

24. A method of deleting a region of vector DNA from a cell according to claim 23, comprising:

a) providing a recombinase activity to the cell; and

b) selecting for cells that lack the desired region of vector DNA.

25. A method of adding a region of DNA to a cell according to claim 23, comprising:

a) introducing the DNA to be added into the cell;

a) providing a recombinase activity to the cell; and

b) selecting for cells that incorporate the added DNA.

26. A method of effecting the inducible expression of a desired gene, comprising:

a) providing a cell according to claim 23 with a recombinase gene that is expressed by an inducible promoter; and

b) inducing said inducible promoter.

27. A method of gene discovery comprising:

a) adding a foreign polynucleotide to a population of target cells such that the foreign

polynucleotide is inserted throughout the genomes of the target cells; and

b) activating control elements encoded by the foreign polynucleotides that activate or repress the expression of target cell genes that flank the integrated foreign polynucleotides, and identifying the regions of the target cell genome into which the foreign polynucleotides have integrated.

28. A library of cultured animal cells that stably integrate vectors according to claims 10 or 11.

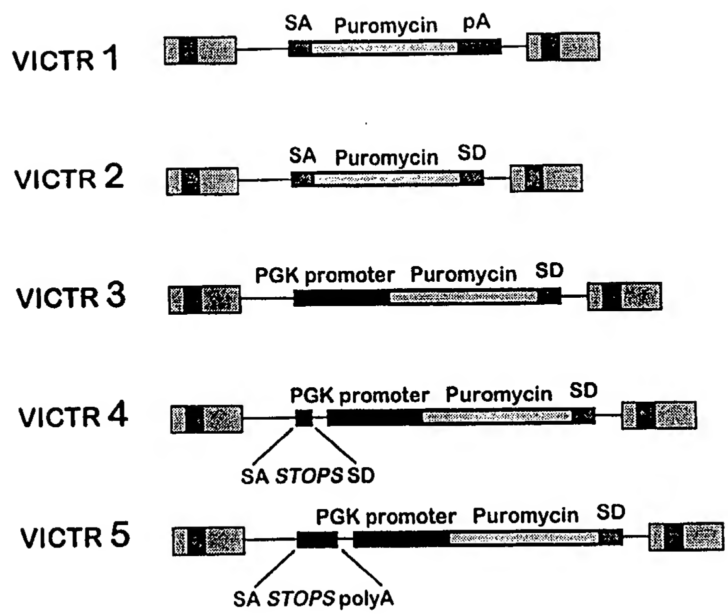

Figure 1

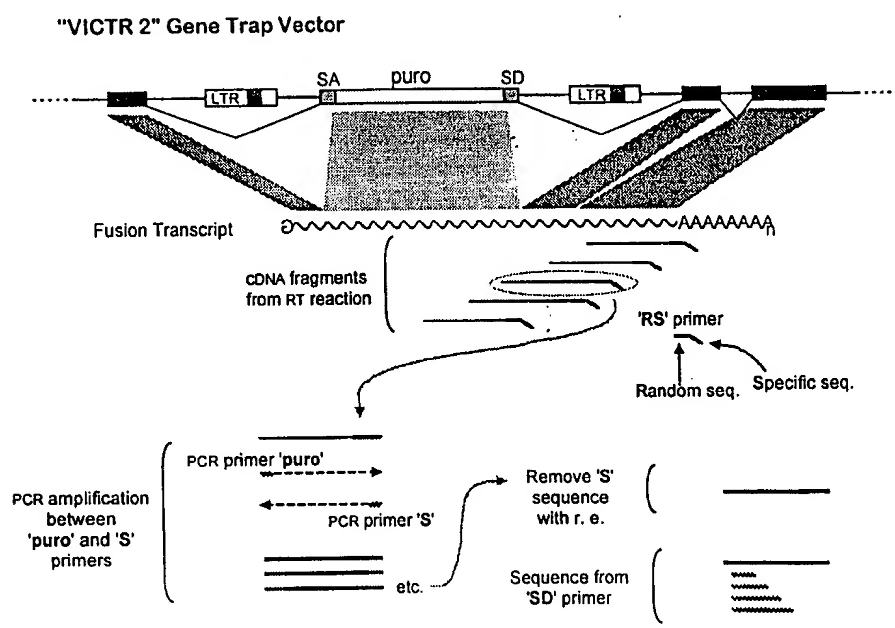

Figure 2

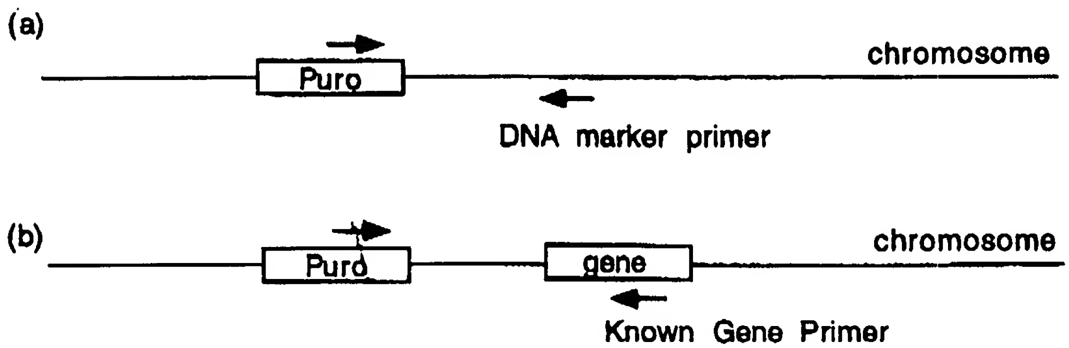
(a)
chromosome
Puro
DNA marker primer
(b)
chromosome
Puro
gene
Known Gene Primer

Figure 3

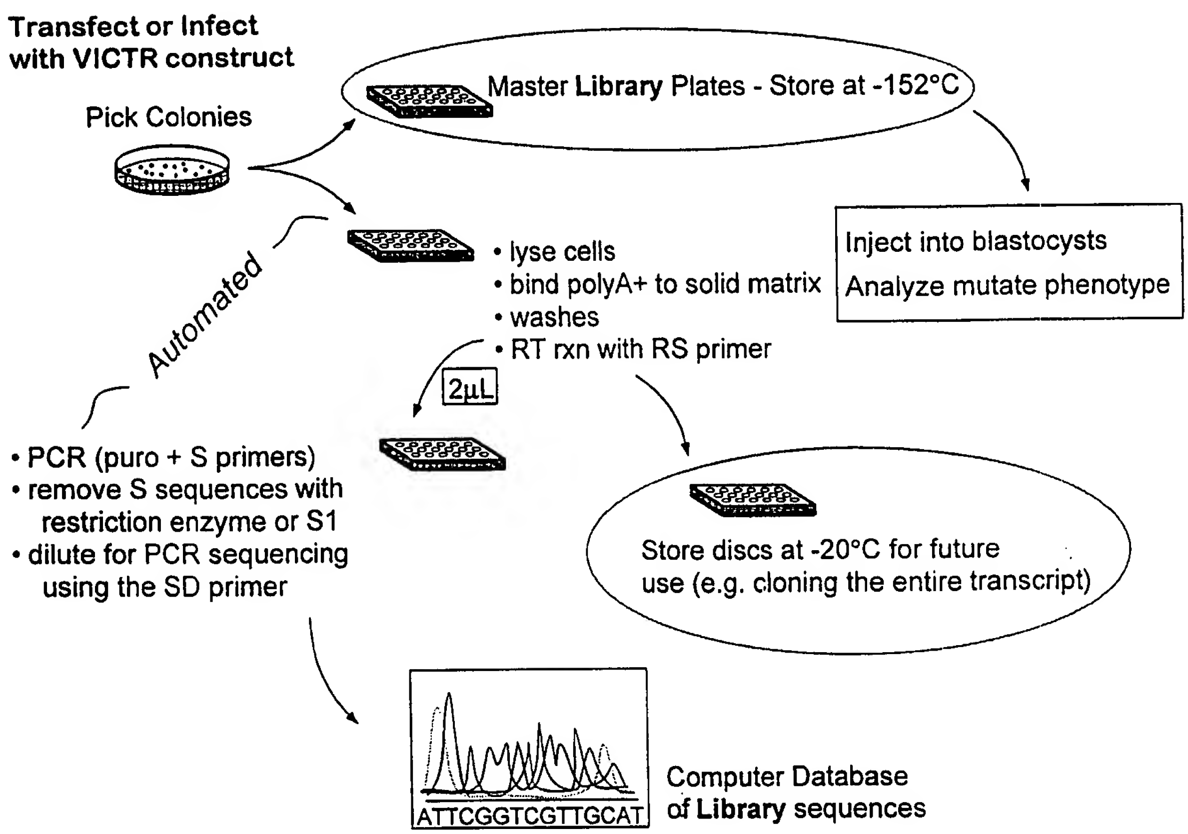
Transfect or Infect
with VICTR construct
Pick Colonies
Master Library Plates - Store at -152°C
Inject into blastocysts
Analyze mutate phenotype
Automated
• lyse cells
• bind polyA+ to solid matrix
• washes
• RT rxn with RS primer
2µL
• PCR (puro + S primers)
• remove S sequences with restriction enzyme or S1
• dilute for PCR sequencing using the SD primer
Store discs at -20°C for future use (e.g. cloning the entire transcript)
ATTCGGTCGTTGCAT
Computer Database
of Library sequences

Figure 4

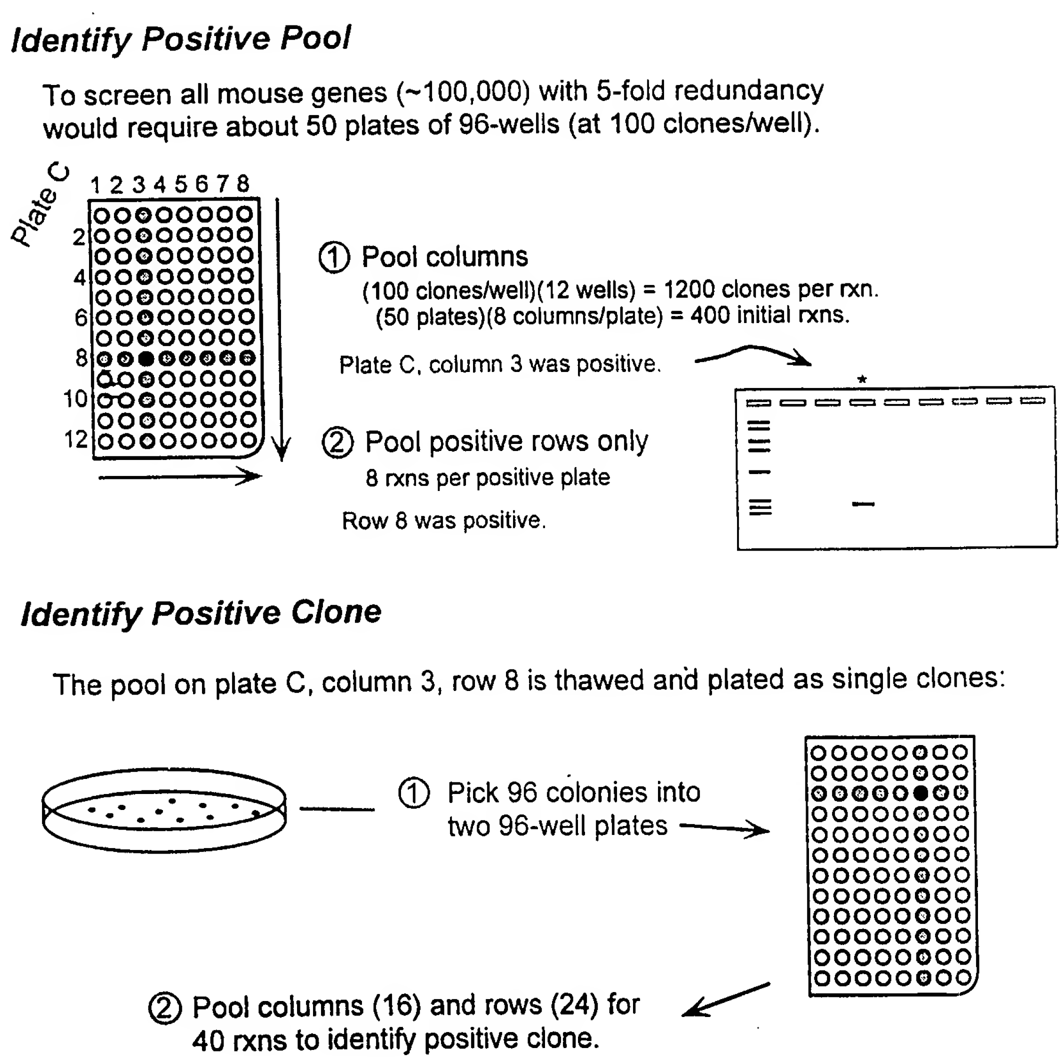
Identify Positive Pool
To screen all mouse genes (~100,000) with 5-fold redundancy would require about 50 plates of 96-wells (at 100 clones/well).
Plate C
1 2 3 4 5 6 7 8
2
4
6
8
10
12
① Pool columns
(100 clones/well)(12 wells) = 1200 clones per rxn.
(50 plates)(8 columns/plate) = 400 initial rxns.
Plate C, column 3 was positive.
② Pool positive rows only
8 rxns per positive plate
Row 8 was positive.
Identify Positive Clone
The pool on plate C, column 3, row 8 is thawed and plated as single clones:
① Pick 96 colonies into two 96-well plates
② Pool columns (16) and rows (24) for 40 rxns to identify positive clone.

Figure 5

```
OST1:                          248 TTTATATAATATTTAATTTGTTTTACTGGGGTATATATGTGTGAAGAGGACTTCT 302
                                   |||| |||||||||||||||||||||||||||||||||||||||||||||||| |
rat GABA rho3:                1547 TTTACATAATATTTAATTTGTTTTACTGGGGTATATATGTGTGAAGAGGACTTTT 1601

OST2:                           56 ACCGTTGCGGAGGCTCACGTTTCTCAGATAGTACATCAGGTGTCATCGNTGTCAGAAGGT 115
                                   |||||||||| | ||||||||||||||||||||||||||||||||||| | |||||| ||
mouse TCR-ATF1:                 75 ACCGTTGCGGGGCCTCACGTTTCTCAGATAGTACATCAGGTGTCATCGTTATCAGAAAGT 134

OST3:                           58 GIGMHHAGLHERDRKTVEELFXNCKVQVLIATSTLAWGVNFPAHLVIIKGTEYYDGKTRR 237
                                   GIG+HHAGL ++DR    +LF   K+Q+LIATSTLAWGVN PAHLVIIKGT+++D K
Yeast ORF G9365:              1430 GIGLHHAGLVQKDRSISHQLFQKNKIQILIATSTLAWGVNLPAHLVIIKGTQFFDAKIEG 1489

OST4:                          137 GCGCAGAAGTGGTNCTGGAANTTTNTCCGCCNCCATCCAGTCTATTAATTGTTGACNGGA 196
seq. from US                       ||||||||||||| ||| || ||| |||||| |||||||||||||||||||||| | |||
patent 5470724:                166 GCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGA 225

OST5:                          108 TCWIRLGT*RXVGASLEYEYIRAS 179
mouse wnt-5A                       TCW++L   R VG +L+ +Y  A+
protein precursor:             250 TCWLQLADFRKVGDALKEKYDSAA 273

OST6:                           78 CTTATATGGCTACGGCGGCTTCAACATCTCCATTACACCCAACTACAGCGTGTCCAGGCT 137
human prolyl                       |||||||||||| |||||||||||||| ||||| |||||||||||||| || ||||||||
endopeptidase:                1407 CTTATATGGCTATGGCGGCTTCAACATATCCATCACACCCAACTACAGTGTTTCCAGGCT 1466

OST7:                          109 AAAGCATGTAGCAGTTGTAGGACACACTAGACGAGAGCACCAGATCTCATTGTGGGTGGT 168
mouse                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
45S pre rRNA:                 1604 AAAGCATGTAGCAGTTGTAGGACACACTAGACGAGAGCACCAGATCTCATTGTGGGTGGT 1663

OST8:                          161 TGGATGCAGNCTACCACTGTGTGGCTGCCCTATTTTACCTCAGTGCCTCAGTTCTGGAAG 220
                                   ||||||||| ||||||||||||||||||||| |||||||||||||||||||| |||||||
rat MAL:                       306 TGGATGCAGCCTACCACTGTGTGGCTGCCCTGTTTTACCTCAGTGCCTCAGTCCTGGAAG 365

OST9:                          103 ACCTGATTGTTATCCGTGGCCTGCAGAAGTCCAGAAAATACAGACCAAAGTCAACCAGTA 162
                                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
mouse malic enzyme:           1666 ACCTGATTGTTATCCGTGGCCTGCAGAAGTCCAGAAAATACAGACCAAAGTCAACCAGTA 1725
```

Figure 6

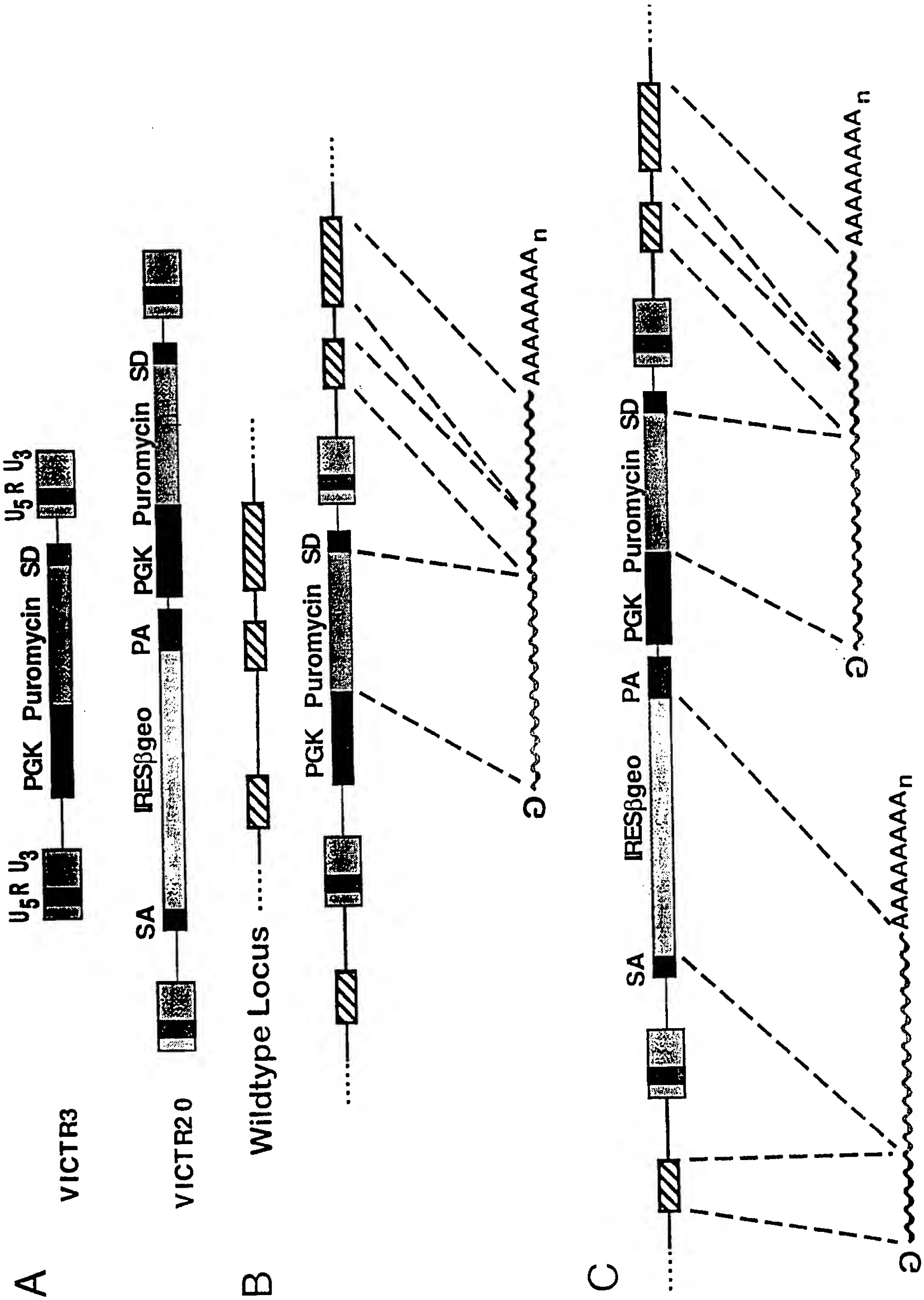
A
VICTR3
U_5R U_3
PGK Puromycin SD
U_5R U_3
VICTR2 0
SA
IRESβgeo
PA
PGK Puromycin SD
B
Wildtype Locus
PGK Puromycin SD
G
$AAAAAAAA_n$
C
SA
IRESβgeo
PA
PGK Puromycin SD
G
$AAAAAAAA_n$
$AAAAAAAA_n$

Figure 7

Figure 8

The following table includes 586 OSTs. OSTs with hit into prodom and GenBank patented sequences have been removed as well as sequence with repetitive elements hits.

OmniBank	DB|Accession	pvalue	Id.	Sequence Description
OST4	gb|W09445	5.0e-133	96%	Mus musculus mu63102.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 315387 5'
OST5	gb|Y00746	2.6e-41	95%	Mus musculus Mouse mRNA for retinal cyclic-GMP phosphodiesterase gamma-subunit (GMP-PDE) (EC 3.1.4.17)
OST22	gb|D88454	5.9e-48	83%	Mus musculus Mouse mRNA
OST25	gb|U28168	1.0e-42	87%	Mus musculus Mus musculus GP106 mRNA, complete cds
OST30	gb|AA048968	1.9e-173	98%	Mus musculus mj50b06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 479507 5'
OST36	gb|D29016	7.5e-71	90%	Mus musculus Mouse mRNA for squalene synthase
OST38	gb|X53732	3.0e-106	95%	Mus musculus M.musculus T cell receptor alpha chain variable region (V-alpha)
OST41	gb|J00360	1.8e-70	10%	Mus musculus mouse alpha-amylase-2 gene: pancreatic mrna
OST42	gb|M33190	4.0e-34	62%	Rattus norvegicus Rat cytochrome P450 II A3 (CYP2A3) gene, complete cds
OST45	gb|AA003309	1.4e-145	99%	Mus musculus mg47d10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 426931 5'
OST51	gb|D86214	1.5e-45	66%	Mus musculus House mouse; Musculus domesticus Postnatal (0 day) Brain mRNA for Ca2+ dependent activator protein for secretion, complete cds
OST56	gb|AA189233	2.6e-37	97%	Mus musculus mu52c11.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 643028 5' similar to TR:G294850 G294850 ALPHA-MUSCLE ACTIN
OST74	gb|Y00169	7.5e-112	89%	Rattus norvegicus Rat TM-4 gene for fibroblast tropomyosin 4
OST75	gb|Z72384	1.0e-126	95%	Mus musculus M.musculus Igk-Vk2(70/3) gene
OST86	gb|AA190122	1.7e-31	88%	Mus musculus mu46f05.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 642465 5'
OST95	gb|AA104745	1.8e-178	96%	Mus musculus mo56d03.r1 Life Tech mouse embryo 8 5dpc 10664019 Mus musculus cDNA clone 557573 5' similar to SW:YA36_SCHPO Q09713 HYPOTHETICAL 37.7 KD PROTEIN C18D11.06 IN CHR1.
OST98	gb|H33806	7.3e-40	88%	Rattus sp. EST110153 Rattus sp. cDNA 5' end
OST117	gb|AA156426	4.0e-111	97%	Homo sapiens zl51b07.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 505429 3' similar to TR:G632498 G632498 CLEAVAGE STIMULATION FACTOR 77KDA SUBUNIT.
OST118	gb|D87684	8.6e-154	84%	Homo sapiens Human mRNA for KIAA0242 gene, partial cds
OST119	gb|D87077	2.0e-145	92%	Homo sapiens Human mRNA for KIAA0240 gene, partial cds
OST121	gb|D28482	3.1e-161	83%	Homo sapiens Human scr2 mRNA for RNA binding protein SCR2, complete cds
OST133	gb|AA114106	1.2e-52	73%	Homo sapiens zn66h09.r1 Stratagene HeLa cell s3 937216 Homo sapiens cDNA clone 563201 5'
OST154	gb|AA107843	4.0e-128	82%	Mus musculus mo49c06.r1 Life Tech mouse embryo 10 5dpc 10665016 Mus musculus cDNA clone 556906 5' similar to gb:J05277 Mouse hexokinase mRNA, complete cds (MOUSE)
OST178	gb|X05300	8.1e-143	92%	Rattus norvegicus Rat mRNA for ribophorin I
OST193	gb|C06148	4.8e-107	84%	Homo sapiens similar to glutamyl-tRNA synthetase
OST243	gb|H32146	4.8e-38	86%	Rattus sp. EST106973 Rattus sp. cDNA 5' end similar to Synapsin I
OST246	gb|AA009152	1.8e-81	79%	Mus musculus mh01b09.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 441209 5'
OST268	gb|M12658	1.2e-91	93%	Mus musculus Mouse 4.5S RNA gene
OST280	gb|AA058245	1.5e-141	94%	Mus musculus mg74e11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 438764 5'

Figure 8 cont'd.

OST281	gb|U65313	1.8e-180	98%	Mus musculus Mus musculus ras-GTPase-activating SH3-domain binding protein (G3BP) gene, complete cds
OST295	gb|AA048190	4.2e-60	83%	Mus musculus mj29a11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 477500 5' similar to gb:J02809 Mouse neural specific calmodulin-binding protein P-57 mRNA,
OST297	gb|Z77585	3.0e-168	10%	Mus musculus M.musculus mRNA for thioredoxin
OST300	gb|M75122	1.8e-203	98%	Mus musculus Mouse acid beta-galactosidase (GLB-1) gene, exon 16
OST301	gb|W34850	2.7e-97	97%	Mus musculus mc62b02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 353067 5' similar to gb:U11248 Mus musculus C57BL/6J ribosomal protein S28 mRNA, complete
OST311	gb|W80427	3.0e-73	85%	Homo sapiens zd82d06.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 347147 3' similar to PIR:A54766 A54766 metastasis-associated protein mta-1
OST314	gb|T34710	4.0e-54	73%	Homo sapiens EST73642 Homo sapiens cDNA 5' end similar to None
OST316	gb|W11499	1.2e-72	99%	Mus musculus ma80h02.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 317043 5' similar to SW:UCRX_BOVIN P00130 UBIQUINOL-CYTOCHROME C REDUCTASE 7.2 KD PROTEIN
OST328	gb|W10861	3.7e-59	89%	Mus musculus ma55c03.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 314596 5'
OST331	gb|U17698	6.8e-119	83%	Mus musculus Mus musculus ablphilin-1 (abi-1) mRNA, complete cds
OST342	gb|U10120	3.1e-143	95%	Mus musculus Mus musculus SKD2 mRNA, complete cds
OST356	gb|M60456	1.8e-117	92%	Mus musculus Mouse cyclophilin mRNA, complete cds
OST361	gb|W77360	5.7e-37	90%	Mus musculus me65f11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 400461 5'
OST368	gb|D87662	2.9e-184	97%	Mus musculus House mouse; Musculus domesticus mRNA for 14-3-3 tau, complete cds
OST386	gb|X99946	2.6e-35	85%	Mus musculus M.musculus 94kb genomic sequence encoding Tsx gene
OST389	gb|T51727	1.8e-78	89%	Homo sapiens yb28c11.s1 Homo sapiens cDNA clone 72500 5'
OST401	gb|W29220	3.1e-33	97%	Mus musculus mc19e08.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 348998 5' similar to SW:YEFA_ECOLI P32054 GDP-D-MANNOSE DEHYDRATASE
OST413	gb|H48542	2.0e-68	78%	Homo sapiens yy49d07.r1 Homo sapiens cDNA clone 276877 5'
OST418	gb|G21163	1.7e-84	85%	Homo sapiens human STS WI-15024
OST421	gb|G25365	6.1e-56	86%	Homo sapiens human STS EST334292
OST425	gb|X04480	8.1e-58	99%	Mus musculus Mouse mRNA for preproinsulin-like growth factor IA
OST430	gb|W97937	5.7e-93	96%	Mus musculus me73g07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 401244 5' similar to gb:M23419 INITIATION FACTOR 5A (HUMAN)
OST439	gb|M26756	2.4e-134	87%	Mus musculus Mouse malic enzyme mRNA, complete cds
OST442	gb|W25938	2.6e-49	70%	Homo sapiens 15b8 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA
OST448	gb|Y07569	4.3e-72	88%	Homo sapiens H.sapiens mRNA for PHAPI2a protein
OST531	gb|X95591	3.1e-206	91%	Mus musculus M.musculus mRNA for CID protein
OST536	gb|W75435	4.6e-75	95%	Mus musculus me50d06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 390923 5' similar to gb:U09659 Mus musculus chaperonin 10 mRNA, complete cds (MOUSE)
OST542	gb|W14808	6.8e-216	99%	Mus musculus mb32g03.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 331156 5' similar to SW:YE04_YEAST P32642 HYPOTHETICAL 27.5 KD PROTEIN IN

				RAD3-BMH1 INTERGENIC REGION. [1]
OST562	gb|X61433	7.6e-68	97%	Mus musculus M.musculus mRNA for sodium/potassium ATPase beta subunit
OST568	gb|AA007930	1.5e-31	67%	Mus musculus mg64a07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 437748 5'
OST571	gb|AA111278	2.1e-147	92%	Mus musculus mo53f02.r1 Life Tech mouse embryo 10 5dpc 10665016 Mus musculus cDNA clone 557307 5'
OST572	gb|AA130347	1.2e-103	85%	Homo sapiens zo05g10.s1 Stratagene endothelial cell 937223 Homo sapiens cDNA clone 566850 3'
OST573	gb|L42855	4.0e-69	75%	Rattus norvegicus Rattus norvegicus RNA polymerase II transcription factor SIII p18 subunit mRNA, complete cds
OST577	gb|AA020459	2.1e-91	92%	Mus musculus mh61a06.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 455410 5'
OST581	gb|R96552	2.0e-90	80%	Homo sapiens yq54e02.r1 Homo sapiens cDNA clone 199610 5'
OST582	gb|D17695	1.9e-218	91%	Rattus rattus Rat mRNA for water channel aquaporin 3 (AQP3), complete cds
OST591	gb|L43326	3.6e-103	85%	Mus musculus Mus musculus domesticus coiled-coil protein (CG-1) mRNA, complete cds
OST593	gb|W70777	3.4e-117	98%	Mus musculus me44a02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 390314 5'
OST594	gb|X94616	2.6e-142	87%	Mus musculus M.musculus mRNA for glycogen synthase
OST595	gb|U67137	7.0e-51	86%	Rattus norvegicus Rattus norvegicus PSD-95/SAP90-associated protein-1 mRNA, complete cds
OST598	gb|X53476	2.2e-235	98%	Mus musculus Mouse mRNA for non-histone chromosomal protein HMG-14
OST600	gb|U70494	1.0e-188	96%	Mus musculus Mus musculus histone H2A.Z (H2A.Z) mRNA, complete cds
OST607	gb|W55702	1.2e-71	85%	Mus musculus ma35d03.r1 Life Tech mouse brain Mus musculus cDNA clone 312677 5'
OST611	gb|AA184809	9.8e-68	97%	Mus musculus mu45h05.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 642393 5' similar to gb:L00993 Mus musculus autoantigen La (MOUSE)
OST618	gb|H11817	1.5e-95	86%	Homo sapiens ym11f07.r1 Homo sapiens cDNA clone 47592 5'
OST620	gb|AA117282	1.0e-78	83%	Mus musculus mn25a10.r1 Beddington mouse embryonic region Mus musculus cDNA clone 538938 5' similar to gb:L35599 Mus musculus Y-box binding protein mRNA, 3' end (MOUSE)
OST623	gb|AA001326	5.7e-106	81%	Homo sapiens zh83b02.r1 Soares fetal liver spleen 1NFLS S1 Homo sapiens cDNA clone 427851 5'
OST626	gb|D83768	1.4e-47	81%	Homo sapiens Human clone N9S Rep-8 mRNA, partial cds
OST663	gb|AA028410	3.2e-114	88%	Mus musculus mi19a06.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 463954 5' similar to gb:M18775 Mouse tau microtubule binding protein mRNA, complete (MOUSE)
OST664	gb|U11027	2.6e-106	87%	Mus musculus Mus musculus C57BL/6J Sec61 protein complex gamma subunit mRNA, complete cds
OST671	gb|S64860	8.4e-211	95%	Mus sp. NonO=non-POU domain-containing octamer-binding protein [mice, B-cell leukemia, BCL1, mRNA, 2411 nt]
OST679	gb|W14516	9.9e-139	95%	Mus musculus mb24h01.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 330385 5'
OST680	gb|L20258	4.2e-232	95%	Mus musculus Mouse stathmin gene
OST702	gb|M78893	5.7e-52	85%	Homo sapiens EST01041 Homo sapiens cDNA clone HHCPB34 similar to cAMP-regulated phosphoprotein
OST707	gb|H19122	1.2e-85	82%	Homo sapiens ym44d11.r1 Homo sapiens cDNA clone 51237 5'
OST716	gb|W62791	4.5e-74	96%	Mus musculus md86e09.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 375304 5'

Figure 8 cont'd.

				(Cx31.1) mRNA, complete cds
OST1096	gb|D87077	7.7e-112	88%	Homo sapiens Human mRNA for KIAA0240 gene, partial cds
OST1105	gb|W44423	1.0e-66	86%	Homo sapiens zc28f04.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 323647 3'
OST1116	gb|X62249	3.1e-36	80%	Mus musculus M.musculus scRNA
OST1137	gb|M63485	1.2e-89	94%	Rattus norvegicus Rat matrin 3 mRNA
OST1145	gb|X80040	1.4e-128	91%	Mus musculus M.musculus Hesx1 mRNA
OST1152	gb|W79970	6.4e-109	10%	Mus musculus me90d10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 402835 5' similar to gb:X57351 INTERFERON-INDUCIBLE PROTEIN 1-8D
OST1155	gb|W10124	2.3e-65	95%	Mus musculus ma42d11.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 313365 5' similar to gb:M29844 APOLIPOPROTEIN C-II PRECURSOR
OST1165	gb|W54649	1.9e-184	97%	Mus musculus md07b12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 367679 5'
OST1179	gb|AA008986	8.5e-84	94%	Mus musculus mg99e02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 441146 5'
OST1186	gb|U89840	2.8e-70	96%	Mus musculus Mus musculus prostate secretory protein (PSP94) mRNA, complete cds
OST1192	gb|W82490	5.3e-127	96%	Mus musculus mf04e04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 404094 5'
OST1207	gb|AA063763	1.3e-56	86%	Mus musculus mj79d10.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 482323 5'
OST1223	gb|AA002931	1.5e-189	99%	Mus musculus mg40f08.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 426279 5'
OST1226	gb|U37353	7.5e-279	95%	Mus musculus Mus musculus protein phosphatase 2A B'alpha3 regulatory subunit mRNA, partial cds
OST1233	gb|W98701	6.3e-91	98%	Mus musculus mg12f05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 423585 5'
OST1234	gb|D85904	1.1e-180	98%	Mus musculus Mouse mRNA for apj-2, complete cds
OST1241	gb|U09850	4.8e-184	92%	Homo sapiens Human zinc finger protein (ZNF143) mRNA, complete cds
OST1247	gb|AA051266	4.7e-126	97%	Mus musculus mj43h02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 478899 5' similar to SW:L10K_RAT Q05310 LEYDIG CELL TUMOR 10 KD PROTEIN
OST1265	gb|X96973	6.8e-183	96%	Mus musculus M.musculus mRNA for Lasp-1 protein
OST1267	gb|R84271	1.6e-32	86%	Homo sapiens yq22g02.r1 Soares retina N2b4HR Homo sapiens cDNA clone 274730 5' similar to SP:YPK1_YEAST P12688 SERINE/THREONINE-PROTEIN KINASE YPK1
OST1269	gb|U19977	4.0e-130	84%	Homo sapiens Human preprocarboxypeptidase A2 (proCPA2) mRNA, complete cds
OST1274	gb|M14634	2.1e-139	85%	Rattus norvegicus Rat mitochondrial propionyl-CoA carboxylase (PCCase) beta-subunit mRNA, complete cds
OST1294	gb|J03583	6.8e-69	95%	Rattus norvegicus Rat clathrin heavy chain mRNA, complete cds
OST1339	gb|M97190	3.1e-118	83%	Homo sapiens Human Sp2 protein mRNA, complete cds
OST1341	gb|W89611	7.8e-142	93%	Mus musculus mf72h02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 419859 5'
OST1354	gb|W74481	2.2e-64	85%	Homo sapiens zd75b12.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 346463 3'
OST1359	gb|W65672	5.1e-35	95%	Mus musculus me13d09.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 387377 5' similar to SW:UBC5_DROME P35128 UBIQUITIN-CONJUGATING ENZYME E2-17 KD
OST1369	gb|W62550	1.1e-109	97%	Mus musculus md73e11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 374060 5' similar to gb:L06039 Mouse platelet endothelial cell
				adhesion molecule 1
OST1376	gb|X63615	1.1e-33	92%	Mus musculus M.musculus Camk-2 mRNA for Ca2+/calmodulin dependent protein kinase II beta subunit
OST1387	gb|C15521	6.5e-57	93%	Homo sapiens Human fetal brain cDNA 5'-end GEN-149C01
OST1410	gb|W59607	4.8e-77	89%	Mus musculus md73a06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 374002 5'
OST1419	gb|W10923	1.2e-122	97%	Mus musculus ma40a11.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 313148 5'
OST1432	gb|W89889	1.7e-145	94%	Mus musculus mf77f07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 420325 5'
OST1453	gb|C18312	5.6e-92	86%	Homo sapiens Human placenta cDNA 5'-end GEN-560D09
OST1457	gb|R78851	5.9e-45	80%	Homo sapiens yi90f03.r1 Homo sapiens cDNA clone 146525 5'
OST1470	gb|J05504	1.5e-136	95%	Mus musculus Mouse guanylate cyclase/atrial natriuretic factor receptor mRNA, complete cds
OST1478	gb|U57821	8.4e-55	80%	Mus musculus Mus musculus inhibitor of MyoD family-b (I-mf) mRNA, complete cds
OST1488	gb|U24681	4.3e-235	94%	synthetic construct Synthetic NADH:cytochrome c reductase fusion protein mRNA, complete cds
OST1492	gb|W10703	3.6e-109	98%	Mus musculus ma53g05.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 314456 5'
OST1493	gb|AA097483	3.5e-197	98%	Mus musculus mk17d04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 493159 5' similar to gb:L05093 60S RIBOSOMAL PROTEIN L18A (HUMAN)
OST1499	gb|T51184	1.3e-60	94%	Homo sapiens yb94h05.r1 Homo sapiens cDNA clone 78873 5'
OST1504	gb|L34260	1.7e-196	91%	Mus musculus Mus musculus integral membrane protein 1 (Itm1) mRNA, complete cds
OST1508	gb|L03306	1.8e-164	94%	Mus musculus Mus musculus core-binding factor mRNA sequence
OST1520	gb|W18420	4.7e-37	88%	Mus musculus mb68e07.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 334596 5' similar to PIR:JH0457 JH0457 80K nuclear protein - human
OST1523	gb|U23769	7.2e-195	92%	Rattus norvegicus Rattus norvegicus CLP36 (clp36) mRNA, complete cds
OST1554	gb|W85270	2.3e-168	96%	Mus musculus mf42d05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 407721 5' similar to SW:IPYR_BOVIN P37980 INORGANIC PYROPHOSPHATASE
OST1556	gb|AA117514	9.7e-150	89%	Mus musculus mn29c09.r1 Beddington mouse embryonic region Mus musculus cDNA clone 539344 5'
OST1558	gb|H33765	2.2e-67	86%	Rattus sp. EST110085 Rattus sp. cDNA 5' end
OST1567	gb|U14636	1.7e-39	94%	Mus musculus Mus musculus serine/threonine protein kinase DLK mRNA, complete cds
OST1601	gb|W39611	1.3e-89	85%	Homo sapiens zc19e08.r1 Soares parathyroid tumor NbHPA Homo sapiens cDNA clone 322790 5'
OST1603	gb|W09922	4.0e-109	86%	Mus musculus ma67e08.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 315782 5' similar to PIR:S48566 S48566 hypothetical protein L8167.23 - yeast
OST1628	gb|U51239	7.9e-78	93%	Mus musculus Mus musculus lysosomal-associated multitransmembrane protein (LAPTm5) mRNA, complete cds
OST1649	gb|AA049090	7.9e-158	96%	Mus musculus mj46e07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 479172 5'
OST1653	gb|AA030795	5.1e-143	97%	Mus musculus mi27f12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 464783 5'
OST1658	gb|W12941	5.2e-95	97%	Mus musculus ma89d07.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone

Figure 8 cont'd.

OST2110	gb|AA051277	6.5e-138	97%	Mus musculus mj43h08.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 478911 5' similar to gb:Y00764 UBIQUINOL-CYTOCHROME C REDUCTASE 11 KD PROTEIN (HUMAN)
OST2112	gb|W85170	4.7e-118	96%	Mus musculus mf43f03.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 407837 5' similar to gb:K00558 TUBULIN ALPHA-1 CHAIN (HUMAN); gb:M13441 Mouse alpha-tubulin isotype M-alpha-6 mRNA, complete cds
OST2116	gb|X76453	2.4e-66	86%	Rattus norvegicus R.norvegicus (Sprague Dawley) H-rev107 mRNA
OST2126	gb|W99867	1.8e-35	85%	Mus musculus mg30b02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 425259 5'
OST2134	gb|W14081	5.2e-109	98%	Mus musculus ma64g01.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 315504 5' similar to PIR:S50866 S50866 4E-BP1 protein - human
OST2141	gb|H91351	3.8e-40	79%	Homo sapiens yu87c07.r1 Homo sapiens cDNA clone 240780 5'
OST2165	gb|W64236	1.7e-144	93%	Mus musculus md95a11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 385724 5'
OST2174	gb|U85055	1.1e-67	93%	Mus musculus Mus musculus rap1/rap2 interacting protein mRNA, complete cds
OST2177	gb|W99250	1.9e-122	86%	Mus musculus mf60h04.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 418711 5'
OST2182	gb|S79780	1.4e-55	93%	Mus sp. DP-3=protein regulating cell cycle transcription factor DRTF1/E2F [mice, p1-2, F9 EC, mRNA, 1380 nt]
OST2188	gb|W10048	2.3e-86	97%	Mus musculus ma67b07.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 315733 5' similar to gb:L02547 CLEAVAGE STIMULATION FACTOR, 50 KD SUBUNIT (HUMAN)
OST2191	gb|U46854	5.4e-61	95%	Mus musculus Mus musculus ShcC mRNA, complete cds
OST2198	gb|T25302	3.1e-33	69%	Homo sapiens EST039R Homo sapiens cDNA clone BL29-39R
OST2218	gb|W38136	4.1e-112	97%	Homo sapiens zc14a11.s1 Soares parathyroid tumor NbHPA Homo sapiens cDNA clone 322268 3'
OST2220	gb|AA049140	6.0e-153	93%	Mus musculus mj49c04.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 479430 5' similar to SW:SYB_TORCA P13701 SYNAPTOBREVIN
OST2229	gb|AA014563	2.8e-109	94%	Mus musculus mi67c05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 468584 5'
OST2236	gb|S63758	2.4e-9[illegible]	96%	Mus sp. metallothionein-1 gene transcription activator [mice, L cells, mRNA, 509 nt]
OST2237	gb|AA002285	1.7e-33	10%	Mus musculus mg42c06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 426442 5' similar to SW:YB81_YEAST P38326 HYPOTHETICAL 34.3 KD PROTEIN IN PDB1-ABD1 INTERGENIC REGION. [1]
OST2250	gb|W71063	2.0e-137	96%	Mus musculus me31e02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 389114 5'
OST2269	gb|U46027	3.1e-119	94%	Mus musculus Mus musculus CREB transcription factor, novel spliced form, mRNA, partial cds
OST2273	gb|U33005	9.9e-111	94%	Mus musculus Mus musculus thel mRNA, complete cds
OST2275	gb|W10226	1.7e-114	95%	Mus musculus mc25h04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 349591 5' similar to SW:CICP_BOVIN P35526 CHLORINE CHANNEL PROTEIN P64.
OST2285	gb|W33930	1.2e-43	92%	Mus musculus mb54c08.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 333230 5' similar to gb:D13315 LACTOYLGLUTATHIONE LYASE (HUMAN)
OST2286	gb|W82037	8.3e-85	96%	Mus musculus me94d11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 403221 5' similar to
				SW:YA9E_SCHPO Q09790 HYPOTHETICAL 23.7 KD PROTEIN C13G6.14 IN CHROMOSOME I.
OST2297	gb|F07734	7.8e-34	86%	Homo sapiens H. sapiens partial cDNA sequence; clone c-2gb07
OST2307	gb|X61399	7.0e-66	92%	Mus musculus Mouse F52 mRNA for a novel protein
OST2321	gb|AA100747	5.1e-85	78%	Homo sapiens zl91h04.r1 Stratagene colon (#937204) Homo sapiens cDNA clone 512023 5'
OST2322	gb|J02811	1.2e-81	92%	Rattus sp. Rat myoadenylate deaminase (AMP deaminase) mRNA, complete cds
OST2346	gb|AB000893	1.5e-95	94%	Mus musculus Mouse mRNA for synaptotagmin 3, complete cds
OST2347	gb|D17653	2.5e-101	85%	Mus musculus Mouse mRNA for HBp15/L22, complete cds
OST2353	gb|T80097	7.4e-73	85%	Homo sapiens yd04g12.r1 Homo sapiens cDNA clone 24692 5'
OST2357	gb|W30066	4.1e-133	99%	Mus musculus mc23a09.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 349336 5'
OST2361	gb|W18873	7.5e-58	95%	Mus musculus mc04d04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 337927 5'
OST2367	gb|X97831	1.9e-104	85%	Rattus norvegicus R.norvegicus mRNA for carnitine/acylcarnitine carrier protein
OST2368	gb|AA013837	6.1e-32	77%	Mus musculus mh24c06.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 443434 5'
OST2379	gb|L10911	1.2e-105	91%	Homo sapiens Homo sapiens splicing factor (CC1.4) mRNA, complete cds
OST2380	gb|W87091	9.5e-88	90%	Mus musculus mf58d10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 418483 5'
OST2381	gb|AA080090	8.3e-126	96%	Mus musculus mj98h06.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 484187 5'
OST2382	gb|AA015380	5.1e-126	92%	Mus musculus mg94g12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 440710 5' similar to gb:M31690 Mouse argininosuccinate synthetase
OST2389	gb|U85614	2.0e-59	91%	Mus musculus Mus musculus SRG3 mRNA, complete cds
OST2395	gb|AA122609	5.0e-138	10%	Mus musculus mn25h06.r1 Beddington mouse embryonic region Mus musculus cDNA clone 539003 5'
OST2400	gb|W34469	4.2e-122	86%	Mus musculus ma98e09.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 318760 5'
OST2401	gb|AA049859	3.7e-83	91%	Mus musculus mj13d01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 475969 5' similar to SW:YAQ6_SCHPO Q10106 HYPOTHETICAL 29.7 KD PROTEIN C18G6.06 IN CHROMOSOME I.
OST2416	gb|W14179	3.1e-54	94%	Mus musculus mb38b08.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 331671 5'
OST2418	gb|N25844	1.3e-47	81%	Homo sapiens yx22f02.r1 Homo sapiens cDNA clone 262491 5'
OST2433	gb|AA104747	2.4e-164	97%	Mus musculus mo56e01.r1 Life Tech mouse embryo 8 5dpc 10664019 Mus musculus cDNA clone 557592 5' similar to gb:M31642 HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE (HUMAN); gb:J00423 mouse hypoxanthine phosphoribosyltransferase(hprt)mrna
OST2442	gb|W35819	1.7e-55	91%	Mus musculus mc14g04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 348534 5'
OST2447	gb|AA061741	1.0e-58	94%	Mus musculus mj90b07.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 483349 5' similar to WP:C32A3.3 CE05343 COILED COIL DOMAINS
OST2455	gb|AA167801	4.2e-62	90%	Homo sapiens zq38c06.r1 Stratagene hNT neuron (#937233) Homo sapiens cDNA clone 631978 5'
OST2459	gb|U05333	3.4e-119	96%	Mus musculus Mus musculus co-chaperonin 'cofactor A' mRNA, complete cds
OST2464	gb|W85263	1.8e-116	94%	Mus musculus mf45h09.r1 Soares mouse

Figure 8 cont'd.

				LPS-binding protein
OST2829	gb|AA002649	7.7e-90	94%	Mus musculus mg38g06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 426106 5' similar to SW:NSE5_HUMAN Q00587 SERUM PROTEIN MSE55. [1]
OST2834	gb|U57692	1.4e-222	97%	Mus musculus Mus musculus N-terminal asparagine amidohydrolase (Ntan1) mRNA, complete cds
OST2835	gb|AA060795	2.1e-89	97%	Mus musculus mj79d05.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 482313 5' similar to WP:F42H10.4 CE00166 CRIP
OST2839	gb|AA163971	6.0e-61	70%	Mus musculus ms40a01.r1 Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone 613992 5'
OST2842	gb|W54515	6.1e-64	91%	Mus musculus md09a10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 367866 5' similar to gb:U07151 ADP-RIBOSYLATION FACTOR-LIKE PROTEIN 3
OST2877	gb|J03583	1.3e-66	93%	Rattus norvegicus Rat clathrin heavy chain mRNA, complete cds
OST2883	gb|W34850	4.8e-75	93%	Mus musculus mc62b02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 353067 5' similar to gb:U11248 Mus musculus C57BL/6J ribosomal protein S28 mRNA, complete (MOUSE)
OST2892	gb|W97758	1.4e-125	98%	Mus musculus mg01e10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 422538 5' similar to gb:J04823_rna1 CYTOCHROME C OXIDASE POLYPEPTIDE VIII-LIVER/HEART
OST2897	gb|W11047	7.9e-132	97%	Mus musculus ma78d10.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 316819 5'
OST2909	gb|AA166258	8.9e-120	96%	Mus musculus ms49c09.r1 Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone 614896 5'
OST2911	gb|U73478	1.4e-117	86%	Mus musculus Mus musculus acidic nuclear phosphoprotein pp32 mRNA, complete cds
OST2914	gb|U12236	4.0e-136	95%	Mus musculus Mus musculus AKR alpha M290 integrin mRNA, complete cds
OST2916	gb|D77002	1.4e-67	92%	Mus musculus Mouse embryonal carcinoma F9 cell cDNA, 93E10
OST2921	gb|W75740	8.4e-106	98%	Mus musculus me55b06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 391379 5'
OST2922	gb|D50544	8.4e-135	88%	Homo sapiens Human lymphocyte mRNA for TFIID subunit p22, complete cds
OST2923	gb|W85631	3.2e-108	97%	Mus musculus mt37b01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 407209 5'
OST2926	gb|W59561	6.3e-164	94%	Mus musculus md72g01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 373968 5'
OST2929	gb|W75735	3.0e-92	92%	Mus musculus me50h12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 390983 5'
OST2934	gb|H82904	1.8e-75	93%	Mus musculus Mouse myotonic dystrophy region mRNA
OST2940	gb|AA154635	1.4e-114	97%	Mus musculus mn44c11.r1 Beddington mouse embryonic region Mus musculus cDNA clone 540788 5' similar to gb:X83590 M.musculus mRNA for ribosomal protein L5, 3'end
OST2942	gb|W34882	1.4e-91	96%	Mus musculus mc40a05.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 350960 5'
OST2948	gb|AA108292	5.1e-32	81%	Rattus norvegicus EST0035 rat lambda ZAPII library (C.P.Hamel) Rattus norvegicus cDNA clone pCO93 5' similar to ADP-ribosylation factor (ARF)-like protein
OST2953	gb|W10606	1.8e-97	98%	Mus musculus ma44d11.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 313557 5' similar to PIR:JS0738 JS0738 ATPase inhibitor protein precursor, mitochondrial - rat [1]
OST2956	gb|AA049172	3.1e-137	97%	Mus musculus mj46d07.r1 Soares mouse

				embryo NbME13.5 14.5 Mus musculus cDNA clone 479149 5' similar to WP:F45E12.4 CE02740
OST2963	gb|W04744	4.2e-31	80%	Homo sapiens za79c08.r1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 298766 5'
OST2971	gb|AA120487	9.2e-107	10%	Mus musculus mn12f07.r1 Beddington mouse embryonic region Mus musculus cDNA clone 537733 5' similar to SW:YDN5_YEAST P38219 HYPOTHETICAL 44.2 KD PROTEIN IN SCO2-MRF1 INTERGENIC REGION.
OST2974	gb|U33553	2.6e-102	88%	Rattus norvegicus Rattus norvegicus neuroglycan C precursor mRNA, complete cds
OST2977	gb|X97755	6.1e-164	97%	Mus musculus M.musculus mRNA MSI
OST2981	gb|AA206420	1.2e-71	85%	Homo sapiens zq51b02.s1 Stratagene neuroepithelium (#937231) Homo sapiens cDNA clone 645099 3' similar to TR:G972006 G972006 MRNA; EXPRESSED SEQUENCE TAG
OST2983	gb|W49206	1.8e-119	98%	Mus musculus mc91g12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 355942 5' similar to PIR:S44900 S44900 ZK652.10 protein - Caenorhabditis elegans
OST2987	gb|AA027683	2.3e-134	96%	Mus musculus mi12b01.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 463273 5'
OST2988	gb|X52129	2.2e-52	73%	Mus musculus domesticus Mouse testis-specific mRNA pBs6.2
OST2989	gb|AA152050	1.3e-46	78%	Homo sapiens z148b12.r1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 505151 5' similar to gb:M90356_cds1 TRANSCRIPTION FACTOR BTF3 (HUMAN)
OST2991	gb|AA003171	8.4e-151	93%	Mus musculus mg56h09.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 437057 5' similar to gb:M24194 GUANINE NUCLEOTIDE-BINDING PROTEIN BETA SUBUNIT-LIKE PROTEIN (HUMAN); gb:X75313 M.musculus
OST2994	gb|R51546	1.9e-51	83%	Homo sapiens yg72h12.r1 Homo sapiens cDNA clone 38905 5' similar to SP:VILI_CHICK P02640
OST2996	gb|X99921	1.6e-82	10%	Mus musculus M.musculus mRNA for S100 calcium-binding protein A13
OST2998	gb|D19012	3.2e-48	10%	Mus musculus Mouse 3'-directed cDNA, MUSGS01209, clone mc0315
OST3003	gb|U27502	1.3e-169	97%	Mus musculus Mus musculus lens major intrinsic protein (MIP) mRNA, complete cds
OST3004	gb|AA103385	1.9e-162	98%	Mus musculus mo23f02.r1 Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone 554427 5' similar to gb:Z15030_rna1 MYOSIN REGULATORY LIGHT CHAIN 2, VENTRICULAR (HUMAN); gb:X65979 M.musculus PLRLC-A mRNA for myosin light chain 2 (MOUSE)
OST3011	gb|AA035805	1.2e-98	99%	Mus musculus mi53a10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 467226 5' similar to PIR:S28237 S28237 NADH dehydrogenase
OST3017	gb|AA050908	4.8e-123	92%	Mus musculus mj21e02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 476762 5' similar to SW:AP17_RAT Q00380 CLATHRIN COAT ASSEMBLY PROTEIN AP17
OST3018	gb|D83277	2.2e-235	99%	Mus musculus Mouse DNA for small GTP-binding protein S10, exon2 and complete cds
OST3032	gb|U49385	2.1e-76	99%	Mus musculus Mus musculus CTP synthetase homolog (CTPSH) mRNA, complete cds
OST3035	gb|L08651	1.8e-115	90%	Mus musculus Mus musculus large ribosomal subunit protein mRNA, complete cds
OST3037	gb|W90956	4.5e-34	74%	Mus musculus mf84h05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 421017 5'

Figure 8 cont'd.

OST3305	gb|D88453	1.0e-106	87%	Mus musculus Mouse mRNA
OST3312	gb|U78109	9.7e-59	66%	Mus musculus Mus musculus prepro-neurturin mRNA, complete cds
OST3323	gb|D43643	1.2e-132	91%	Mus musculus Mouse YL-1 mRNA for YL-1 protein (nuclear protein with DNA-binding ability), complete cds
OST3324	gb|X61399	2.2e-51	87%	Mus musculus Mouse F52 mRNA for a novel protein
OST3325	gb|D28476	6.5e-103	94%	Homo sapiens Human mRNA for KIAA0045 gene, complete cds
OST3349	gb|M18210	2.2e-52	94%	Mus musculus Mouse transcription factor S-II, clone PSII-3
OST3352	gb|AA099569	4.9e-63	77%	Homo sapiens zk86b04.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 489679 3'
OST3354	gb|W53638	9.1e-69	92%	Mus musculus md14d10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 368371 5' similar to SW:RNG3_HUMAN P25440 RING3 PROTEIN. [1]
OST3355	gb|U49185	4.1e-40	82%	Mus musculus Mus musculus occludin mRNA, complete cds
OST3366	gb|AA122835	2.1e-85	69%	Mus musculus mn24g03.r1 Beddington mouse embryonic region Mus musculus cDNA clone 538900 5' similar to gb:D00682 COFILIN (HUMAN); gb:D00472 Mouse mRNA for cofilin, complete cds and flanks (MOUSE)
OST3370	gb|S67058	4.6e-106	94%	Mus sp. Hoxa-4/Hox-1.4=Hoxa-4 [mice, Genomic, 2556 nt]
OST3371	gb|W31107	1.5e-50	71%	Homo sapiens zb85e12.r1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 310414 5'
OST3372	gb|W64859	2.2e-134	99%	Mus musculus me06f10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 386731 5' similar to PIR:A55012 A55012 signal peptidase 25k chain - dog
OST3375	gb|AA015237	4.0e-44	10%	Mus musculus mh30a10.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 443994 5'
OST3376	gb|M27347	4.2e-103	99%	Mus musculus Mus musculus p6-5 gene, 3' end
OST3388	gb|D50264	1.9e-117	98%	Mus musculus Mouse mRNA for phosphatidylinositol glycan class F, complete cds
OST3390	gb|W34022	3.6e-46	78%	Mus musculus mb01d09.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 318929 5'
OST3393	gb|U60330	1.7e-208	93%	Mus musculus Mus musculus Ki antigen mRNA, complete cds
OST3404	gb|AA168895	6.3e-109	98%	Mus musculus ms41g02.r1 Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone 614162 5' similar to gb:M23419 INITIATION FACTOR 5A
OST3413	gb|N91837	3.3e-39	91%	Homo sapiens zb45f03.s1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 306557 3'
OST3425	gb|W71116	1.3e-105	88%	Mus musculus me31d05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 389097 5'
OST3428	gb|AA189339	3.4e-37	88%	Mus musculus mt79g04.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 636150 5'
OST3441	gb|S51858	7.9e-66	77%	Mus sp. MO25 gene [mice, embryos, mRNA, 2322 nt]
OST3450	gb|X58426	7.1e-53	96%	Mus musculus Mouse mRNA for hepatic triglyceride lipase
OST3457	gb|W87064	9.0e-166	97%	Mus musculus mf58h05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 418521 5'
OST3460	gb|AA185211	4.7e-134	99%	Mus musculus mu51g08.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 642974 5' similar to TR:E243948 E243948 CHROMOSOME VII READING FRAME ORF YGL054C.
OST3480	gb|AA118567	9.4e-100	89%	Mus musculus mn12a08.r1 Beddington mouse embryonic region Mus musculus cDNA clone 537686 5'
OST3481	gb|X56906	1.0e-121	95%	Mus musculus Mouse OP-1 mRNA for osteogenic protein 1

OST3483	gb|X79446	1.4e-114	92%	Mus musculus M.musculus Odf1 mRNA for outer dense fiber protein of sperm tails
OST3485	gb|D83824	1.4e-75	86%	Homo sapiens similar to T cell-specific MAL
OST3492	gb|W09518	4.7e-139	92%	Mus musculus ma08d09.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 303953 5'
OST3494	gb|W61666	1.1e-138	99%	Mus musculus md82d01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 374881 5' similar to SW:GBG5_BOVIN P30670 GUANINE NUCLEOTIDE-BINDING PROTEIN G(I)/G(S)/G(O) GAMMA-5 SUBUNIT. [1]
OST3500	gb|U62483	2.1e-180	98%	Mus musculus Mus musculus ubiquitin conjugating enzyme (ubc4) mRNA, complete cds
OST3501	gb|D59851	6.8e-54	90%	Homo sapiens Human fetal brain cDNA 5'-end GEN-070H03
OST3505	gb|W40883	3.9e-173	99%	Mus musculus mc39d07.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 350893 5'
OST3508	gb|M23458	2.0e-119	90%	Mus musculus Mus musculus endogenous retroviruslike B-26 (distantly related to MulV) LTR
OST3516	gb|L14441	5.4e-177	90%	Rattus norvegicus Rat phosphotidylethanolamine N-methyltransferase mRNA, complete cds
OST3517	gb|AA015044	5.5e-114	97%	Mus musculus mh23f10.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 443371 5'
OST3518	gb|AA061165	6.3e-99	91%	Mus musculus mj31f05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 477729 5' similar to TR:E222933 E222933 SUPEROXIDE DISMUTASE
OST3521	gb|H33756	3.7e-70	87%	Rattus sp. EST110066 Rattus sp. cDNA 3' end
OST3531	gb|U19893	6.7e-34	80%	Rattus norvegicus Rattus norvegicus alpha actinin mRNA, complete cds
OST3534	gb|U37150	5.7e-31	83%	Bos taurus Bos taurus peptide methionine sulfoxide reductase (msrA) mRNA, complete cds
OST3545	gb|M93148	4.0e-103	84%	Mus musculus Mouse homeobox protein (Hox-1.11) gene, complete cds
OST3556	gb|W08748	1.9e-129	97%	Mus musculus mb48f02.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 332667 5'
OST3558	gb|L03386	7.9e-132	97%	Rattus norvegicus Rattus norvegicus (clone RAHB2-5/8) zinc finger protein mRNA, 3' end cds
OST3561	gb|W13785	5.1e-64	99%	Mus musculus ma94c11.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 318356 5' similar to SW:RS27_RAT P24051 40S RIBOSOMAL PROTEIN S27. [1]
OST3567	gb|AA050004	2.8e-48	78%	Mus musculus mj39d07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 478477 5'
OST3571	gb|W75236	2.4e-113	91%	Mus musculus me53a07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 391188 5'
OST3575	gb|AA080212	6.0e-90	93%	Mus musculus mj99a06.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 484210 5' similar to gb:X58079 S-100 PROTEIN, ALPHA CHAIN (HUMAN)
OST3579	gb|S74622	1.1e-39	76%	Gallus gallus PP1M M21 subunit=protein phosphatase 1M 21 kda regulatory subunit [chickens, gizzard smooth muscle, mRNA, 1598 nt]
OST3582	gb|X74350	1.5e-74	99%	Mus musculus M.musculus XPAC Xerderma Pigmentosum group A Correcting gene, exon 6
OST3601	gb|U50078	4.6e-138	89%	Homo sapiens Human guanine nucleotide exchange factor p532 mRNA, complete cds
OST3602	gb|R15062	2.3e-107	90%	Homo sapiens yf86h05.r1 Homo sapiens cDNA clone 29483 5'
OST3604	gb|M22756	4.9e-119	84%	Rattus norvegicus Rat 24-kDa subunit of mitochondrial NADH dehydrogenase mRNA, 3' end
OST3608	gb|U34994	5.4e-101	85%	Homo sapiens Human DNA-dependent

Figure 8 cont'd.

				protein kinase catalytic subunit (DNA-PKcs) mRNA, complete cds
OST3609	gb|AA165901	2.4e-129	96%	Mus musculus mc75e03.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 635740 5'
OST3631	gb|AA028590	2.1e-152	97%	Mus musculus mi21c12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 464182 5' similar to WP:R01H2.6 CE00805 UBC
OST3642	gb|H85211	1.4e-47	74%	Homo sapiens yo41d11.s1 Homo sapiens cDNA clone 180501 3' similar to SP:S19586 S19586 N-METHYL-D-ASPARTATE RECEPTOR GLUTAMATE-BINDING CHAIN -
OST3645	gb|M14951	1.3e-104	90%	Mus musculus Mouse insulin-like growth factor II (IGF-II) mRNA, complete cds
OST3647	gb|U14721	1.7e-36	76%	Mus musculus Mus musculus c-abl oncogene (c-abl) gene, exons 2 and 3, partial cds
OST3651	gb|AA023146	1.4e-109	91%	Mus musculus mh67b03.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 455981 5' similar to SW:A4P_HUMAN Q04941 INTESTINAL MEMBRANE A4 PROTEIN. [1]
OST3652	gb|S60494	3.1e-31	94%	Mus sp. gamma-phosphorylase kinase (alternatively spliced) [mice, muscle, Balb/C, Genomic, 4204 nt, segment 4 of 4]
OST3662	gb|U37427	3.1e-204	96%	Rattus norvegicus Rattus norvegicus phospholipid hydroperoxide glutathione peroxidase mRNA, complete cds
OST3669	gb|W55918	3.0e-35	86%	Homo sapiens zc03f12.s1 Soares parathyroid tumor NbHPA Homo sapiens cDNA clone 321263 3' similar to WP:E04F6.2 CE01214
OST3681	gb|W55833	7.6e-94	93%	Mus musculus md07b01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 367657 5' similar to gb:D37874 Mouse FcRn gene. (MOUSE)
OST3694	gb|W38194	5.4e-71	93%	Homo sapiens zc15e05.s1 Soares parathyroid tumor NbHPA Homo sapiens cDNA clone 322400 3'
OST3700	gb|AA038243	4.9e-171	99%	Mus musculus mi82d08.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 473103 5' similar to SW:SARL_RABIT P42532 SARCOLIPIN. [1]
OST3703	gb|W47847	7.8e-71	82%	Mus musculus mc82f12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 355055 5'
OST3704	gb|AA048648	4.6e-68	99%	Mus musculus mj33a07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 477876 5'
OST3708	gb|AA002275	7.4e-89	97%	Mus musculus mg43h01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 426577 5' similar to gb:M73704 PHOSPHATIDYLINOSITOL (HUMAN)
OST3716	gb|AA034685	8.2e-119	90%	Mus musculus mi56h10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 467587 5' similar to gb:L19527 60S RIBOSOMAL PROTEIN L27
OST3729	gb|W19303	2.9e-97	85%	Homo sapiens zb25d02.r1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 303075 5'
OST3731	gb|W11502	1.3e-131	93%	Mus musculus ma80h06.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 317051 5' similar to SW:PRCF_HUMAN P40306 PROTEASOME COMPONENT MECL-1 PRECURSOR
OST3735	gb|AA014575	5.2e-100	97%	Mus musculus mi67g07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 468636 5' similar to SW:SYRM_YEAST P38714 ARGINYL-TRNA SYNTHETASE, MITOCHONDRIAL PRECURSOR
OST3757	gb|W77924	2.6e-99	83%	Homo sapiens zd71f04.r1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 346111 5'
OST3759	gb|X64840	7.6e-51	97%	Mus musculus M.musculus ALF1 mRNA
OST3767	gb|C18536	5.2e-39	69%	Homo sapiens Human placenta cDNA 5'-end GEN-563H06
OST3775	gb|D18282	1.6e-57	97%	Mus musculus Mouse 3'-directed cDNA, MUSGS00768, clone md0842

OST3788	gb|AA014426	9.7e-55	10%	Mus musculus mg84b01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 439657 5' similar to SW:NB7M_BOVIN Q02367 NADH-UBIQUINONE OXIDOREDUCTASE B17 SUBUNIT
OST3789	gb|D13544	9.5e-67	97%	Mus musculus Mouse mRNA for primase small subunit, complete cds
OST3807	gb|W26968	3.8e-51	80%	Homo sapiens 16h7 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA
OST3818	gb|M28248	3.8e-48	96%	unidentified cloning vector Moloney murine leukemia virus retroviral vector pLXSN, complete genome
OST3819	gb|T55632	3.8e-35	81%	Homo sapiens yb39b03.r1 Homo sapiens cDNA clone 73517 5' similar to SP:T20G5.10 CE00629
OST3827	gb|AA046830	1.2e-67	84%	Homo sapiens zf12h11.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 376773 3'
OST3831	gb|W70777	3.5e-121	99%	Mus musculus me44a02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 390314 5'
OST3839	gb|H86008	1.4e-103	86%	Homo sapiens EST02533 Homo sapiens cDNA clone HFUCY19 similar to Hypothetical 43.5K protein
OST3843	gb|Z82190	2.8e-51	88%	Homo sapiens Human DNA sequence *** SEQUENCING IN PROGRESS *** from clone 180M12; HTGS phase 1
OST3849	gb|W64986	1.3e-173	94%	Mus musculus me04c05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 386504 5' similar to SW:VSH7_DICDI P14327 VEGETATIVE SPECIFIC PROTEIN H7. [1]
OST3851	gb|U51037	1.0e-135	84%	Mus musculus Mus musculus 11-zinc-finger transcription factor (CTCF) mRNA, complete cds
OST3858	gb|X56135	4.7e-237	97%	Mus musculus Mouse mRNA for prothymosin alpha
OST3864	gb|D19493	9.8e-33	95%	Mus musculus Mouse 3'-directed cDNA, MUSGS00881, clone mb0610
OST3869	gb|W41525	4.4e-100	85%	Mus musculus mc45b04.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 351439 5'
OST3897	gb|W10485	3.8e-97	95%	Mus musculus ma53e06.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 314434 5'
OST3903	gb|W59388	1.2e-108	86%	Mus musculus md79f02.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 374619 5' similar to gb:U07151 ADP-RIBOSYLATION FACTOR-LIKE PROTEIN 3 (HUMAN)
OST3905	gb|D85430	8.0e-102	92%	Mus musculus Mouse Murr1 mRNA, exon
OST3909	gb|AA020459	1.2e-80	94%	Mus musculus mh61a06.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 455410 5'
OST3917	gb|Z44044	8.7e-81	87%	Homo sapiens H. sapiens partial cDNA sequence; clone c-1rc07
OST3924	gb|J04699	3.9e-32	84%	Mus musculus Mouse nicotinic acetylcholine receptor beta subunit (nAChRE) gene, complete cds
OST3925	gb|W23511	1.2e-88	76%	Homo sapiens zb46e02.r1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 306650 5'
OST3931	gb|U14957	1.6e-36	81%	Homo sapiens Human 53K isoform of Type II phosphatidylinositol-4-phosphate 5-kinase (PIPK) mRNA, complete cds
OST3945	gb|W15004	1.6e-122	97%	Mus musculus mb25c09.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 330448 5'
OST3957	gb|AA051293	2.8e-143	96%	Mus musculus mj40h10.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 478627 5' similar to SW:TGFB_HUMAN P22064 TRANSFORMING GROWTH FACTOR BETA-1 BINDING PROTEIN PRECURSOR
OST3960	gb|D38614	1.1e-88	82%	Mus musculus Mouse 921-S mRNA for presynaptic protein, complete cds
OST3961	gb|U67988	6.6e-37	77%	Homo sapiens Human guanylate kinase associated protein (GKAP) mRNA, complete cds

Figure 8 cont'd.

OST3971	gb|W45926	9.6e-55	94%	Mus musculus mc79e04.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 354750 5'
OST3988	gb|H13524	2.6e-111	90%	Mus musculus Mouse serum amyyloid A pseudogene (psi-SAA)
OST3993	gb|R16778	4.7e-45	82%	Homo sapiens yf33a08.s1 Homo sapiens cDNA clone 128630 3'
OST4002	gb|AA000314	1.9e-112	96%	Mus musculus mg34e07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 425700 5'
OST4003	gb|L37297	2.9e-121	91%	Mus musculus Mus musculus (clone B6) myeloid secondary granule protein mRNA
OST4011	gb|L26664	2.0e-155	94%	Mus musculus Mus musculus expressed sequence tag EST F032
OST4028	gb|D87470	7.5e-93	92%	Homo sapiens Human mRNA for KIAA0280 gene, partial cds
OST4033	gb|AA084704	2.2e-54	88%	Homo sapiens zn05f04.s1 Stratagene hNT neuron (#937233) Homo sapiens cDNA clone 546559 3' similar to TR:G600529 G600529 NADH UBIQUINONE OXIDOREDUCTASE SUBUNIT
OST4051	gb|F03500	7.6e-63	86%	Homo sapiens H. sapiens partial cDNA sequence; clone c-1zd08
OST4061	gb|W30618	3.1e-118	97%	Mus musculus mc10h12.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 348167 5'
OST4070	gb|W36515	6.0e-135	94%	Mus musculus mb76g12.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 335398 5'
OST4073	gb|X82021	2.0e-105	91%	Rattus norvegicus R.norvegicus mRNA for heat shock related protein
OST4074	gb|D63704	3.3e-140	86%	Rattus norvegicus Rat mRNA for dihydropyrimidinase, complete cds
OST4106	gb|W75804	1.1e-84	93%	Mus musculus me67a06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 400594 5'
OST4114	gb|W20730	6.5e-90	96%	Mus musculus mb96g01.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 337296 5'
OST4131	gb|AA044274	2.4e-33	69%	Homo sapiens zk54h03.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 486677 3'
OST4134	gb|H31489	3.0e-84	85%	Rattus sp. EST105564 Rattus sp. cDNA 3' end
OST4140	gb|W71052	3.7e-121	91%	Mus musculus me27f01.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 388729 5' similar to SW:YBH8_YEAST P38182 HYPOTHETICAL 13.6 KD PROTEIN IN PET112-ILS1 INTERGENIC REGION. [1]
OST4142	gb|C07091	5.7e-74	89%	Rattus norvegicus similar to none
OST4144	gb|X56135	4.4e-41	83%	Mus musculus Mouse mRNA for prothymosin alpha
OST4148	gb|W54510	1.5e-138	91%	Mus musculus md08h09.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 367841 5' similar to PIR:A56059 A56059 protein-tyrosine-phosphatase
OST4149	gb|U36393	2.6e-111	96%	Mus musculus Mus musculus transcription factor TFEB mRNA, partial cds
OST4154	gb|X56046	1.3e-161	96%	Mus musculus Mouse mRNA (clone lambda-16) for hypothetical protein A
OST4155	gb|X05900	3.5e-58	85%	Rattus norvegicus Rat mRNA for lens betaB1-crystallin (pRLbeta B1-3)
OST4166	gb|U53859	8.0e-169	90%	Rattus norvegicus Rattus norvegicus calpain small subunit (css1) mRNA, partial cds
OST4174	gb|U41395	1.3e-38	84%	Mus musculus Mus musculus X inactive specific transcript (Xist) gene, cosmid MB4-14A, fragment 2
OST4191	gb|X63507	2.0e-75	81%	Mus musculus M.musculus HOX-3.5 gene
OST4192	gb|W85357	2.2e-83	82%	Mus musculus mf49h12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 408455 5' similar to SW:GLYM_HUMAN P34897 SERINE HYDROXYMETHYLTRANSFERASE, MITOCHONDRIAL
OST4194	gb|W34635	8.9e-38	87%	Mus musculus mc31e07.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 350148 5'

OST4196	gb|W41301	3.1e-39	99%	Mus musculus mc43h06.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 351323 5'
OST4223	gb|AA203787	2.7e-89	90%	Mus musculus mu60f12.r1 Soares mouse lymph node NbMLN Mus musculus cDNA clone 643823 5'
OST4228	gb|S51016	9.3e-205	92%	Bos taurus E2(25K)=multiubiquitinating enzyme [cattle, thymus, mRNA, 825 nt]
OST4229	gb|Z31263	4.8e-70	97%	Mus musculus M.musculus expressed sequence tag MTEST7
OST4235	gb|W53187	3.0e-173	97%	Mus musculus md19a07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 368820 5' similar to WP:C32D5.9 CE01849
OST4243	gb|AA048921	2.3e-40	86%	Mus musculus mj47e11.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 479276 5' similar to gb:U13705 Mus musculus domesticus C57BL/6J plasma glutathione (MOUSE)
OST4245	gb|H10216	9.9e-80	75%	Homo sapiens ym02f05.s1 Homo sapiens cDNA clone 46710 3'
OST4247	gb|AA023146	1.5e-115	96%	Mus musculus mh67b03.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 455981 5' similar to SW:A4P_HUMAN Q04941 INTESTINAL MEMBRANE A4 PROTEIN. [1]
OST4251	gb|AA070774	8.7e-154	98%	Homo sapiens zm53g11.s1 Stratagene fibroblast (#937212) Homo sapiens cDNA clone 529412 3'
OST4254	gb|W54737	2.4e-82	10%	Mus musculus md10a04.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 367950 5'
OST4258	gb|AA013789	4.3e-169	90%	Mus musculus mh13d03.r1 Soares mouse placenta 4NbMP13.5 14.5 Mus musculus cDNA clone 442373 5' similar to PIR:JC2472 JC2472 RE protein - human
OST4281	gb|U16175	4.0e-40	63%	Mus musculus Mus musculus thrombospondin 3 (Thbs3) gene, partial cds and mucin 1 (Muc1) gene, complete cds
OST4283	gb|AA007519	8.9e-52	81%	Homo sapiens zh98e12.r1 Soares fetal liver spleen 1NFLS S1 Homo sapiens cDNA clone 429358 5'
OST4288	gb|AA000024	1.4e-135	96%	Mus musculus mg33e06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone 425602 5' similar to gb:X03920_rna2 M.musculus GSHPx gene (MOUSE)
OST4315	gb|M18210	6.4e-62	96%	Mus musculus Mouse transcription factor S-II, clone PSII-3
OST4319	gb|J04696	2.0e-127	95%	Mus musculus Mouse glutathione S-transferase class mu (GST5-5) mRNA, complete cds

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C12N 5/02, 5/06, 15/00, 15/64; C07H 21/04
US CL :435/6, 320.1, 325, 357; 536/23.1, 24.2; 800/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 320.1, 325, 357; 536/23.1, 24.2; 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAUER, B. Site-specific recombination; developments and applications. Current Opinion in Biotechnology. May 1994, Vol. 5, pages 521-527, see the entire article.	1-8, 10, 20 and 28
Y	SEKINE et al. Frameshifting is required for production of the transposase encoded by insertion sequence 1. Proc. Natl. Acad. Sci. USA. June 1989, Vol. 86, pages 4609-4613, see especially "Frameshifting in Other Systems", page 4613.	10
X	WANG, et al. High frequency recombination between loxP sites in human chromosomes mediated by an adenovirus vector expressing Cre recombinase. Somatic Cell and Molecular Genetics. 09 March 1996, Vol. 21, No. 6, pages 429-441, see especially the abstract.	8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 JANUARY 1998	02 MAR 1998

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	WILLIAM SANDALS
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ODELL et al. Site-directed recombination in the genome of transgenic tobacco. Molecular and General Genetics. 11 October 1990, Vol. 223, pages 369-378, see especially Figure 1 and the "Result" section.	1-8, 10, 20
X	DYMECKI, S. A modular set of Flp, FRT and LacZ fusion vectors for manipulating genes by site-specific recombination. Gene. 01 June 1996, Vol. 171, pages 197-201, see especially Figure 1.	10
X	HAAS et al. TnMax - a versatile mini-transposon for the analysis of cloned genes and shuttle mutagenesis. Gene. 11 August 1993, Vol. 130, pages 23-31, see especially the abstract.	8
Y	WO 88/01646 (ALLELIX INC.) 10 March 1988 (10.10.88), see especially pages 1-3.	1-8, 10 and 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8, 10, 20 and 28

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-7, 8, 10, 20 and 28, drawn to a library of cultured eucaryotic cells made by a process comprising treating a group of cells with a vector that mediates the splicing of a foreign exon internal to a cellular transcript, the use of the cell from the library to generate a non-human transgenic animal, and the method of making the cell comprising the vector and the use of the vector to make the library of cultured eukaryotic cells.

Group II, claim(s) 9, 11-18, drawn to a vector construct for replacing the 3' end of an animal cell transcript with a foreign exon.

Group III, claim(s) 19, 21 and 22, drawn to the use of a vector according to claim 9.
Group IV, claim 23, drawn to a stably transduced animal cell that incorporates the vector of claim 16.
Group V, claims 24-27, drawn to a method of altering a region of DNA by adding or deleting DNA.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the first group contains the product, a library of cultured eukaryotic cell, a method of using the cells to produce a non-human transgenic animal and a method of making the cells. The additional groups are directed to different vectors having different compositions than the vector used in the first group, cell lines containing those vector constructs and methods of altering the cellular genome. The first group contains a vector having a different composition than the other vectors and therefore the special technical feature present in the first group does not occur in the other groups.